Hallmarks of senescence and aging

Slavica Dodig*1, Ivana Čepelak¹, Ivan Pavić²

¹Department of Medical Biochemistry and Hematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
²Department of Pulmonology, Allergology and Immunology, Children’s Hospital Zagreb; School of Medicine, University of Zagreb, Zagreb, Croatia

*Corresponding author: slavica.dodig@zg.t-com.hr

Abstract
The complex process of biological aging, as an intrinsic feature of living beings, is the result of genetic and, to a greater extent, environmental factors and time. For many of the changes taking place in the body during aging, three factors are important: inflammation, immune aging and senescence (cellular aging, biological aging). Senescence is an irreversible form of long-term cell-cycle arrest, caused by excessive intracellular or extracellular stress or damage. The purpose of this cell-cycle arrest is to limit the proliferation of damaged cells, to eliminate accumulated harmful factors and to disable potential malignant cell transformation. As the biological age does not have to be in accordance with the chronological age, it is important to find specific hallmarks and biomarkers that could objectively determine the rate of age of a person. These biomarkers might be a valuable measure of physiological, i.e. biological age. Biomarkers should meet several criteria. For example, they have to predict the rate of aging, monitor a basic process that underlies the aging process, be able to be tested repeatedly without harming the person. In addition, biomarkers have to be indicators of biological processes, pathogenic processes or pharmacological responses to therapeutic intervention. It is considered that the telomere length is the weak biomarker (with poor predictive accuracy), and there is currently no reliable biomarker that meets all the necessary criteria.

Keywords: senescence; aging; biomarkers; hallmarks

Received: February 25, 2019 Accepted: June 10, 2019

Introduction
In the past two decades the field of both aging and senescence research has undergone a significant progress. Aging can be defined as the time-relating irreversible proliferative deterioration of those physiological processes of the organism that support its survival and fertility (1). The result of aging processes is the progressive loss of physiological integrity and impaired function of tissues and organs. With prolonged human lifespan, aging also moves towards the older age. Recently, elderly age was classified into three periods: elderly or early old age, senile or middle old age and late old age (or long-livers). Early old age ranging from 60 to 75 years is the period of initial involution of human physical capabilities. Then follows the middle old age, from 76 to 90 years, the period of further involution of human motor functions. Finally, after 90 years of age, a late old age is following; it is a period of decline in human physical abilities (2).

Every living organism lives in a permanent struggle with extrinsic and intrinsic agents that can damage it. Without its own repair mechanisms, life of living creatures would be extremely short, since the accumulation of harmful substances would damage the cellular elements, their function, which would ultimately result in damage to the various tissues and accelerated aging of the entire organism.

Most of the aging definition involves a gradual, heterogeneous impair in the structure, function,
and maintenance of repair systems of various organs and an increased inclination to various diseases. One could say that the age/aging phases are easy to recognize, but the mechanisms responsible for the aging process are difficult to define and harder to prove. Technological progress has established various methodological approaches to detect some cellular and molecular mechanisms associated with aging. Among others, scientists have focused recently on senescence (cellular aging, biological aging) mechanisms as one of the key factor in a complex aging process (3,4).

This review focuses on human senescence and aging processes, and their mechanisms. Particular attention was directed to hallmarks of these processes and their possible biomarkers. In search of scientific and review papers on the PubMed free search engine, the following key words were used: lifespan, aging, systems biology, senescence, hallmark, markers of aging, biomarkers, markers of senescence, senescence testing, and bioinformatics. Epidemiological and clinical researches were studied primarily on older people, regardless of their ethical affiliation. Also, animal models of aging investigation were studied. Abstracts, reports from meetings and case control studies were excluded. Articles published in English between 1997 and 2019 were included. Articles were selected according to relevance to the topic.

Three different responses that have protective role in response to cellular stressors are apoptosis (programmed cell death), autophagy (from the Greek noun „autóphagos”, meaning self-devouring) and senescence (irreversible arrest, that limits the proliferation of damaged cells) (5-8). It seems that the cellular response depends on the type of cell that is subjected to the harmful effect of the stressor. While damaged lymphocytes tend to undergo apoptosis, damaged epithelial cells and fibroblasts tend to undergo senescence (5). Autophagy implies a lysosome-mediated cell’s own components bulk degradation and clearance (5,9). The relationship between autophagy and apoptosis is complex. It is not yet clear which factor determines whether cells will die with apoptosis or with other mechanisms. It seems that autophagy could be conducive to cell death in cases when apoptosis is inhibited (5). While activation of autophagy causes inhibition of apoptosis, its inhibition increases susceptibility to oxidative damage of the cell and apoptosis. Prolonged autophagy is associated with cell death. Autophagy becomes defective during ageing and especially in patients with age-related diseases, since degraded molecules and organelles accumulate in cells. Hence, defective autophagy is a feature of old cells (7). Schematic depiction of the aging process, with possible therapeutic interventions is shown in Figure 1.

### Senescence

Senescence (from the Latin word „senex“, meaning growing old) is an irreversible form of long-term cell-cycle arrest, caused by excessive intracellular or extracellular stress or damage (12). In order to avoid malignant transformation after the stressor’s activity, cellular senescence refers to the arrest in the G1 phase of the cell-cycle (5). Senescent cells are however functionally and metabolic active as changes occur, for example change of degradation pathways of proteins, enhanced mitochondrial metabolism, energy generation, etc. (13). The purpose of senescent cells arrest is to limit the proliferation of damaged cells (e.g. the spread of damage to the next cell generation), to eliminate accumulated harmful factors and to disable potential malignant transformation (5-8). In young tissues, transient senescence has beneficial effect. The good example is the beneficial effect of senescence to pregnancy that implies proper foetal development and time of parturition. A detrimental effect refers to reproductive capacity since it causes the decrease in the number of ovarian follicles, and in later age senescence causes decline in ovarian and uterine function (14). Healthy senescence may be accelerated by elevating the concentration of oxygen or various toxic substances (15). Factors that slow down damage accumulation delay the senescence.

Based on kinetics of cell senescent processes there are two main categories of senescence, i.e. acute (transient) and chronic (persistent) senescence (16). Acute senescence is the part of normal biological processes, and has beneficial effect within
Figure 1. Overview of the process of senescence and its contribution to aging of entire organism (adapted according to references 5, 10 and 11). Based on kinetics of cell senescent processes there are two main categories of senescence – acute (programmed, transient) and chronic (not programmed, persistent) senescence. While acute senescence leads to embryonic development, wound healing and tissue repair of specific populations of cells and tissues, chronic senescence that is not directed towards specific cells leads into a stable cell-cycle arrest, a state that limits the proliferation of damaged cells. The main mediator of acute senescence is SASP. It seems that, because of age-related immunodeficiency or less production of proinflammatory SASP factors, immune cells becomes inefficiently in the elimination of senescent cells. p53, p16 and other tumour suppressor pathways mediators leads to senescence. Cancer development will occur if pre-senescent cells (stressed cells) would not been removed by specific mechanisms. However, it is not known which mechanisms are responsible for direction to senescence, apoptosis or to autophagy. Production of SASP factors may be inhibited by the use of: nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), interleukine 1α blockers, rapamycin, metformin; senescent cell killing may be induced by natural killer cells, T cell targeting, antibodies or antibody-mediated drug delivery. Early in life, senescent cells are transiently present and have a beneficial effect on development, homeostasis, and regeneration. However, at a later age, senescent cells accumulate and produce detrimental effects. ROS – reactive oxygen species. SASP – senescence-associated secretory phenotype. p53 – cellular tumour antigen p53. p21 – cyclin-dependent kinase inhibitor 1, cell-cycle inhibitor. p16 – cyclin-dependent kinase inhibitor 2A, multiple tumour suppressor 1.
tissues during embryonic development, wound healing or tissue repair. Myofibroblasts have an important role during acute senescence, because they promptly undergo senescence, limiting excessive fibrosis at the site of cell/tissue damage. Acute senescence may be a part of programmed mechanism of fibrosis control during tissue repair (17). Acute senescent cells are eliminated through activation of senescence-associated secretory phenotype (SASP) factors and consequently activated immune clearance. Senescent cells, still metabolically active, found primarily in tissues with chronic inflammation and in renewable tissues, are able to create an inflammatory microenvironment, to recruit phagocytic cells for elimination of senescent cells and finally, to promote tissue removal. They secrete a variety of different molecules to communicate with adjacent cells. Senescence is enabled with the acquisition of SASP factors, such as interleukins (the most prominent is interleukin-6, IL-6), chemokines, growth factors (e.g. insulin-like growth factor, IGF) and regulators, proteases (e.g. matrix metalloproteinases - MMPs, serine proteases), etc. (8,18,19). Released SASP factors are involved in sensitizing non-senescent neighbouring cells to senesce, cell proliferation, disruption of normal tissue structure and function, immunomodulation (immune cells clearance), angiogenesis, disabling or fostering of cancer growth. SASP factors have beneficial role during embryogenic development, accelerating wound healing, after tissue injury (by limiting fibrosis), involved in the amplification and spread of senescent cells, during suppression of tumorigenesis by promoting the elimination of senescent cells. The main function of SASP is to eliminate senescent cells. If there were no senescent cell clearance as in case in elderly people, senescent cells would accumulate, which would have detrimental consequences implying structural, degenerative, irreparable tissue damage and fibrosis (7,20). Chronic senescence is induced through prolonged period of cellular stress or slow macromolecular damage (10,16). Complex effector pathways involved in chronic senescence significantly differ from pathways in acute senescence, due to large SASP heterogeneity involved in chronic processes and high resistance of senescent cells to immune clearance. Chronic senescence has detrimental effects within cells and tissues. The knowledge that senescence can cause age-related diseases has instigated researchers to develop drugs that can eliminate senescent cells. These medications could improve health in the elderly (Figure 1) (11,20).

Senescent cells in elderly are not able to maintain neither physiological tissue functions nor tissue repair, including autophagy, whose capacity declines with aging (7,21,22). Cellular senescence is followed by senescent cell clearance within those processes that are considered beneficial. However, if the elimination of senescent cells does not occur, senescent cells accumulate and can lead to cancer and aging. Investigations on animal samples have shown that senescent cells accumulate in old animals in leukocytes and intestinal crypt enterocytes, in dermal fibroblasts, hepatocytes, osteocytes (23).

Unlike apoptosis in which phagocytes remove cells without causing inflammation, senescent cell survive because of stimulation of the inflammatory environment and removal of harmful compounds (24). Senescence-associated beta-galactosidase (SA-β-GAL), is an isoform of the beta-galactosidase enzyme, normally responsible for the breakdown of beta-galactosides. Its activity is present in lysosomes of senescent cells. Increased activity of SA-β-GAL is considered to be an outcome of senescence (7).

Factors leading to senescence

Senescence can be triggered e.g. by oxidative stress, telomere damage/shortening, DNA damage, mitochondrial dysfunction, chromatin disruption, inflammation, epigenetic dysregulation, and oncogene activation (17,25-27).

Oxidative stress

It is known that senescent phenotype may be stimulated/induced by various types of stresses, including that induced by reactive oxygen species (ROS). Reactive oxygen species are a natural by-product of the normal oxygen metabolism. It is considered that ROS regulate several physiological
functions, like signal transduction, gene expression and proliferation. The major cellular sources of ROS are mitochondria, cell membranes and endoplasmic reticulum (28). While lengthening of organismal lifespan is associated with low ROS concentration, senescent phenotype maintenance is endangered with high ROS concentrations (29). The oxidant/antioxidant imbalance causes a structural damage of macromolecules (DNA, proteins and lipids). Age-related accumulation of damaged macromolecules is one of mechanisms that contribute to the aging processes. The balance between oxidant generation and antioxidant processes in healthy tissues is maintained with a predominance of various antioxidants (30,31).

Reactive oxygen species of endogenous or exogenous origin induce and firm the senescent phenotype by a process that involves the response to DNA damage, epigenetic regulation and tumour suppression pathway activation (e.g. cell cycle control related proteins: p53 (cellular tumour antigen p53), p21 (p21Cip1, cyclin-dependent kinase inhibitor 1), pRB (retinoblastoma protein). These mechanisms, more specifically SASP factors of senescent cells, on the other hand, can stimulate positive feedback loop and result in increased ROS, especially mitochondrial ROS (mtROS) (32). As mitochondria are the main place of ROS creation, investigations have shown that mitochondrial dysfunction is associated with senescence, and consequently with the aging process. It is considered that mtROS and oxidative stress in general can stimulate telomeres shortening and dysfunction, which is one of the characteristics of aging (33). In addition to ROS, as senescence inducers, other mitochondrial-related effectors are also considered, for example, redox changes, changed metabolism (34,35).

Telomere shortening

Telomeres (from the Greek nouns „telos“ meaning end and „meros“ meaning part), specialized DNA-protein structures of human chromosomes, composed of several kilobases (kb) of simple repeats (TTAGGG)n are located at the ends of chromosomes. The length of telomeres is an accurate predictor of the replicative ability of cells. The basic function of telomeres is to protect the chromosomes from degradation rearrangements, end-to-end fusions, and chromosome loss (36). Shortening occurs at each cellular division but is counteracted by telomerase. Telomerase is an enzyme complex that maintains telomere length. It is considered that telomeres participate in the protection of ends of chromosomes from constitutive exposure to the DNA damage response (37). Telomere length progressively shortens with replication of nuclear DNA during mitosis, or with oxidative stress or with senescence and aging (38).

While the length of the telomere at birth is about 11 to 15 kb in elderly it is significantly shorter, about 4 kb (39-42). So, senescence is mostly triggered when the length of the telomere shorten from 5–20 kb to 4–7 kb (43). The shortening of the telomeres that occurs during normal aging is controlled by the activity of specialized enzyme telomerase (27). However, the balance between telomere shortening and counteracting by telomerase is disrupted during accelerated senescence as a result of the disease.

DNA damage

Critically short telomeres are recognized as DNA damage, which trigger a DNA damage response (DDR). The DDR arrests cell cycle progression until damages are repaired. However, senescent cells display persistent DDR foci that are resistant to endogenous DNA repair (44).

Mitochondrial DNA damage

Mitochondria are intracellular source of oxygen. Functional mitochondria regulates cellular homeostasis through the maintenance of redox balance, which implies a balance between oxygen uptake, ATP production, membrane potential and generation of ROS (45). Mitochondria that accumulate in senescent cells show increased concentrations of ROS and increased rate of senescent cells in the same tissues, resulting in mitochondrial dysfunction (27,45).

Tumour suppressors and cell cycle inhibitors

Today, several suppressors and cell cycle inhibitors are known, e.g. p16 (known as cyclin-dependent
kinase inhibitor 2A, multiple tumour suppressor 1), p53, p21, p15 (p15INK4b, protein kinase; cyclin-dependent protein serine/threonine kinase inhibitor, multiple tumour suppressor), p27 (cyclin-dependent kinases regulator), ADP-ribosylation factor (ARF), hypophosphorylated retinoblastoma protein (7,11). Activation of the tumour suppression pathways p53 and p21 and the p16/retinoblastoma protein pathways occurs during senescence. Activation is triggered by the DNA damage, which may be result of telomeric and non-telomeric DNA damage or oxidative stress (27).

**Characteristics of senescent cells**

Senescent cells are characterised by flattened and enlarged morphology. They exhibit several molecular markers, including telomere-dysfunction-induced foci, senescence-associated heterochromatin foci (SAHF), lipofuscin granules, DNA scars, altered gene expression (5,7). Another important feature of senescent cells is release of SASP factors (19). As the senescent cells are characterized by the irreversible growth arrest in either G₁ or G₂/M phase of the cell cycle, they are no longer able to divide. These cells have special biochemical characteristics, e.g. the absence of proliferative Ki-67 protein, activity of senescence-associated β-galactosidase (SA-β-GAL), expression of tumour suppressors and cell cycle inhibitors (7,11). Nuclear and mitochondrial DNA damage accelerate senescence. As long as the repair mechanisms are effective, the cell damage can be repaired. Otherwise, when some of the repair mechanisms fail, damaged DNA will accumulate, obstructing cellular function and causing its senescence. Inducers of senescence, such as telomere shortening, toxic agents or oncogenes, cause the formation of SAHF, that contain heterochromatin-forming proteins, such as heterochromatin protein 1 (HP1) proteins, di- or tri-methylated lysine 9 of histone H3 (H3K9Me2/3) and histone H2A variant (macroH2A) (46,47). All these cellular characteristics can be considered as hallmarks (or possible biomarkers) of senescence.

**Aging**

Aging has been the focus of researchers for many years. Scientists are trying answer two basic questions on biochemical level: „Why does human being (and all living organisms) age?” and „How do organisms age?”. Consequently, there are a large number of aging theories that are classified in a variety of ways. For example, one of classifications theories includes the evolutionary and causality theories (48). Evolutionary aging theories, that are focused on the failure of natural selection to affect late-life traits, refer to programmed aging (assisted death), non-programmed aging and senemorphic aging (maladaptive aging, secondary aging). Causality theories imply the influence of the environmental conditions on cellular senescence and ultimate death. The main role was given to telomeres shortening, free radicals damages, spontaneous errors, glycation end-products (48). There are also theories that attempt to explain the aging process itself - on the one hand there are theories considering the senescence as programmed processes; other theories, e.g. „DNA damage theory of aging” are focused on the accumulation of damage as the main cause of biological aging (22,49).

Aging is an intrinsic feature of all living beings. The complex process of biological aging is the result of genetic and, to a greater extent, environmental factors and time. It occurs heterogeneously across multiple cells and tissues. As the rate of aging is not the same in all humans, the biological age does not have to be in accordance with the chronological age. Many age-associated changes and hallmarks are evident in the human body. The changes associated with old age can be divided into a few categories: normal aging, somatic diseases and multiple chronic conditions, psychological, cognitive and social changes (50). Normal aging implies sensory changes (visual acuity, hearing loss, dizziness), muscles weakening and reduced mobility ability, fat changes. At the same time the body increasingly succumbs to some diseases, including hypertension, cardiovascular diseases, diabetes, osteoarthritis, osteoporosis, cancer, and several neurological disorders. In elderly there are several functional changes of respiratory system such as reduction of vital capacity, increased residual volume, reduction of pulmonary diffusion, increased arterial-alveolar oxygen gradient, hypoxia, hypercapnia, increased percent of neutrophil
granulocytes, increased ratio of CD4+/CD8+ cells in bronchoalveolar lavage fluid and decreased level of antioxidant compounds (i.e. superoxide dismutase, glutathione, catalase, metal binding proteins, vitamins C and E) (51,52). In addition, there is a decreased number of functional glomeruli, decreased rate of glomerular filtration and renal blood flow (53). Occurrence of electrolytic disturbances (e.g. hyper- or hyponatremia) may worse other comorbidities (54). Also, there is a decrease in basal metabolism, the change in gastrointestinal system, as well as in the hypothalamic-pituitary-adrenal systems. The later results with low response to stimulation of this axis (54). In the background of all the changes that occur during aging are three key factors – inflammation, immune aging and senescence.

**Inflammation and aging**

Unlike acute (transient) inflammation in which the causative agents are removed and the damaged tissue is cured, chronic inflammation persists for a long time. During chronic inflammation affected tissues are infiltrated with macrophages and lymphocytes. In addition, fibrous and necrosis of the affected tissue may occur (18,55). Chronic inflammation is associated with many age-related physiologic or pathophysiologic processes and diseases. In normal, healthy aging, serum concentrations of pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, IL-22, IL-23, tumour necrosis factor alpha – TNF-α, and interferon-gamma – IFN-γ) are significantly increased in comparison with younger individuals (56-58). At the same time, in elderly people concentration of anti-inflammatory cytokines (interleukin-1 receptor antagonist – IL-1Ra, IL-4, IL-10, IL-37, transforming growth factor beta 1 – TGF-β1) are higher than in young persons. The role of anti-inflammatory cytokines is to neutralize pro-inflammatory cytokine activity, reduce chronic inflammation, and thus act protectively on tissues. In the case of healthy aging, a balance between the action of pro-inflammatory and anti-inflammatory mediators has been established. Their imbalance leads to aging of the body and to the development of various age-related pathological conditions (59).

**Immune system and aging**

The weakening of unspecific innate and highly specific acquired immunity takes place through the aging of human cells (Table 1). The phagocytic function is reduced, while, chemotaxis may be conserved, especially in the presence stimulants of the complement fragment C5a (57). The number of macrophage precursors is decreased, the phagocytic function is reduced, neutrophil dysfunction is observed, and naive lymphocytes produce less IL-2, the number of CD8+ lymphocytes increases. The senile age is characterized by a high expression of CD25 and FOXP3 (a transcriptional factor that is crucial for the function of Treg cells), and increased number of CD4+/FOXP3 lymphocytes, changed T17/Treg ratio. All these changes are responsible for the appearance of inflammatory and autoimmune diseases (60). Impaired NK function of natural killers (NK) is associated with an occurrence of infective, atherosclerotic and neurodegenerative diseases. As the thymus exhibits degenerative changes, impaired function of both, B cells and T cells leads to imbalance between inflammatory and anti-inflammatory mechanisms. Frequent infectious diseases in old age are a result of impaired function of the innate and acquired immune system. Immune system fails to clear infectious antigens, infected cells, senescent cells, and malignant transformed cells (56,61). Immunological changes in elderly, based on the decline of the functional capacity of the immune system, result in reduced resistance to infections, increased appearance of neoplasia, and increased production of auto-antibodies responsible for the occurrence of autoimmune diseases (62).

As individuals of the same age do not have the same rate of age, there is a need to find specific hallmarks that could objectively determine the rate of age of a person. These biomarkers might be a valuable measure of physiological/biological age. Still, there is no universally accepted definition of a biomarker of aging. Phenotypic hallmarks are non-invasive biomarkers, and easy to obtain (Table 2). Biochemical biomarkers can reflect some of the biochemical mechanisms underlying age status. It would be ideal if quantitative aging biomarkers could specifically determine the biologi-
Senescence and aging testing

In order to examine why and how people become old with different rate, it is necessary to define the primary indicators/biomarkers of the healthy aging process. Only in this way it will be possible to distinguish the phenomenon of aging due to the processes caused by various diseases that are commonly associated with the aging process. In this sense, the scientific community is continually investing great efforts in discovering such biomarkers.

In general, a biomarker is defined as any substance, structure or process that can be objectively measured in the body or its products and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention (68,69). Thus, there are diagnostic, prognostic, predictive and pharmacodynamic biomarkers.

According to the American Federation for Aging Research (AFAR) recommendations, aging biomarkers should meet several criteria. They have to: 1. predict the rate of aging (correlate with aging); 2. monitor a basic process that underlies the aging process (determine “healthy aging”, not the effects of disease); 3. be able to be tested repeatedly without harming the person; 4. be applicable to humans and animals (70). However, currently, there is no biomarker that would meet all of these criteria. Scientific papers refer at biomarkers of senescence (or senescent cells) as well as at aging biomarkers. Currently, due to the stated fact that many of the hallmarks do not meet biomarker definition criteria.

### Table 1. Features of immune aging

| Cell                     | Features                                                                 |
|--------------------------|--------------------------------------------------------------------------|
| Innate immunity          |                                                                          |
| Neutrophils              | Reduced phagocytosis and ROS production                                  |
| Monocytes/Macrophages    | Reduced phagocytosis, cytokine and chemokine secretion, reduced generation of NO and superoxide, reduced IFN-γ, inhibited response to growth factors |
| Dendritic Cells          | Reduced phagocytosis and pinocytosis, increased IL-6 and TNF-α production, diminished TLR expression and function |
| Eosinophils              | Reduced degranulation and superoxide production                          |
| Cytotoxic lymphocytes    |                                                                          |
| NK                       | Reduced numbers, increased reduced numbers, reduced cytotoxicity         |
| NKT                      | Reduced proliferation                                                    |
| Acquired immunity        |                                                                          |
| B cells                  | Decreased number, reduced proliferative capacity, increased oligoclonal expansion, reduced surface MHC class II molecule expression, reduced antibody avidity, increased concentration of IgG, IgA and concentration of autoantibodies |
| T cells                  | Reduced CD28 expression, accumulation of CD8+CD28+ T cells, reduced TCR diversity, reduced signal transduction, reduced response and proliferation, increased differentiation of CD4+ into Th17 cells |
| Treg                     | Increased CD8+FOXP3+, decreased CD8+CD45RA+CCR7+                        |

ROS - reactive oxygen species. NO - nitric oxide. NK – natural killer cells. NKT – natural killer T cell. Treg – T-regulatory cells. TCR – T-cell receptor. IL – interleukine. IFN – interferon γ. TLR – toll-like receptor. TNF-α – tumour necrosis factor α. MHC – major histocompatibility complex. CD – cluster of differentiation. FOXP - transcription (factor) protein. CCR – chemokine receptor. Adapted according to references 63-66.
### Table 2. Phenotypic and biochemical hallmarks of aging

| Hallmark category | Hallmark subcategory | Hallmark | Trend during aging |
|-------------------|----------------------|----------|--------------------|
| **Phenotypic**    | Anthropometry and physical function | BMI, waist circumference | I |
|                   | Facial features       | Eye corner slope | D |
|                   |                       | Nose width, Mouth width, Noise-mouth distance | I |
|                   |                       | Mouth width | I |
|                   |                       | Noise-mouth distance | I |
| **Biochemical**   | Nutrient sensing      | (S/P) Growth hormone and IGF-1 | D |
|                   | Protein metabolism    | (S/P) Protein carbamylation, e.g. homocitruline rate | I |
|                   |                       | (Erc) Glycosated hemoglobin | I |
|                   | (S/P) Advanced glycation end products N-glycans | I |
| **Lipid metabolism** |                       | (S/P) Lipid profile, free fatty acids, isoprostanes | I |
| **Oxidative stress** |                       | (Erc) superoxide dismutase | D |
|                   |                       | (Erc) glutathione, glutathione reductase, glutathione peroxidase | HD |
| **Hormone, energy** |                       | (S/P) Triiodothyronine, cortisol | D |
| **Inflammation**  |                       | (S/P) C-reactive protein, interleukin 6 | I |
| **Organ-specific** | Cardiovascular system | (S/P) troponin, natriuretic peptides, endothelin | I |
|                   | Lung                  | (S/P) surfactant protein D | I |
|                   |                       | (arterial blood) partial pressure of oxygen | D |
|                   | Kidney                | (S/U) Glomerular filtration rate | D |
|                   |                       | (S/P) creatinine, urea | I |
|                   | Liver                 | (S/P) ALT, AST, GGT, albumin | D |
|                   | Reproductive function | (S/P) LH, FSH, DHEA | D |
|                   | Oxygen transport      | (B) Htc, Hb, MCV, Rtc | D |
|                   |                       | (S) erythropoietin, ferritin, hepcidin | D |
|                   | Blood clotting        | (S/P) D-dimers | I |
|                   |                       | (B) platelet count | D |
|                   |                       | (Pilt) platelet functions | I |
|                   |                       | (P) Fibrinogen | I |

BMI – body mass index. IGF-1 – insulin-like growth factor 1, somatomedin C. S/P – serum/plasma. Erc – erythrocytes. S/U – serum/urine. B – blood. S – serum. P – plasma. ALT – alanine aminotransferase. AST – aspartate aminotransferase. GGT – gamma-glutamyl transferase. LH – luteinizing hormone. FSH – follicle-stimulating hormone. DHEA – dehydroepiandrosterone. Htc – haematocrit. Hb – haemoglobin. MCV – mean cell volume. Rtc – reticulocytes. I – increased. D – decreased. HD – increased in elderly hypertensive patients treated for their conditions. Adapted according to reference 70.

It may be better to use terms a) hallmarks of senescent cells or hallmarks of aging or b) possible biomarkers of senescence. 
Research on why and how the senescence goes on should shed more light on this intriguing process.

The corresponding biomarker can be identified either in pro-senescent mechanisms either in anti-senescent pathways. Different methods for detection of senescence in tissue sections or in cultured cells (fibroblasts) are used (Table 3). It is possible to
Table 3. Laboratory methods used for determination of possible senescent-cell biomarkers

| Analyte                                      | Method                                      | References |
|----------------------------------------------|---------------------------------------------|------------|
| morphological analysis                       | inverted phase-contrast microscope          | 73         |
| cell viability                               | tetrazolium reduction, microplate spectrophotometer | 71         |
| SASP                                         | ELISA                                       | 12,68      |
| SAHF                                         | immunohistochemistry                        | 12         |
| γH2AX                                        | histochrometry                              | 12,68      |
| p16, p53, and p21                            | histochrometry, immunohistochemistry        | 12         |
| SA-β-GAL                                     | histochrometry, immunohistochemistry, flow cytometry | 12,68,79   |
| autophagy                                    | immunoblotting                             | 72         |
| cell proliferation                           | flow cytometry                             | 73         |
| leukocyte absolute telomere length           | southern blot, PCR, FISH                    | 68,75,76   |

ELISA – enzyme linked immunosorbt assay. SASP – senescence-associated secretory phenotype. SAHF – senescence-associated heterochromatin foci. γH2AX – a type of histone protein from the H2A family, a marker for activation of DNA damage response. PCR – polymerase chain reaction. p16 – cyclin-dependent kinase inhibitor 2A, multiple tumor suppressor 1. p53 – tumour suppressor gene, induces senescence growth arrest via activated p21–p53 pathway. p21 – cell-cycle inhibitor, induces senescence growth arrest via activated p21–p53 pathway. SA-β-GAL – senescence-associated β-galactosidase. FISH – fluorescent in situ hybridization.

A lysosomal hydrolase, SA-β-GAL, normally active at pH 4, in senescent cells is active at pH 6. However, the SA-β-GAL, is present not only in senescent cells but also in presenescent, quiescent or immortal cells (77). It may be detected in tissue sections histochemically and immunohistochemically (12,78). Conventional SA-β-GAL staining fails to distinguish between different cell types that can be a source of senescent cells within complex tissues, limiting our understanding of the underlying biological phenomena. (73,79). As the single parameter is not enough to define with confidence that cells are senescent, SA-β-GAL staining may be combined with additional possible biomarkers, e.g. SASP factors, SAHF formation, γH2AX (a type of histone protein from the H2A family, a marker for activation of DNA damage response), p16, p53 (induces senescence growth arrest via activated p21–p53 pathway), and p21 concentrations (induces senescence growth arrest via activated p21–p53 pathway) (12). Telomere attrition is the intrinsic property of healthy cellular aging, and is also associated with many age-related diseases, like atherosclerosis, myocardial infarction, heart failure, Alzheimer’s dementia (76). For more than a decade telomere length has (most often average leukocyte telomere length) been postulated as a biomarker of human aging (80).

These possible biomarkers are detected separately in consecutive sections; it means that multiple possible biomarkers are not determined within the same cells. Although it was confirmed in mouse tissues that most possible markers increase with age, there is still insufficient data that would refer to healthy human tissues (77). Telomere length measurement is emerging as a tool that may have implications for prevention, disease monitoring, and intervention development. It has been a subject of debate whether telomere length is a biomarker of aging in specific tissues or for a whole organism, since the aging of different tis-
sues and organs of the human body is not the same (3,81). Therefore, in human aging, telomere length is a weak biomarker with poor predictive accuracy. Glycans might be a better possible biomarker of chronological and biological age than telomere lengths (81,82). Histochemical staining of lipofuscin (i.e. lipid - containing lysosomal granules) of paraffin sections has been shown to be one of the possible markers of senescence in agerelated diseases (83,84). Recently a new method for the determination of lipofuscin in liquid samples of stressed or damaged cells was introduced (85). Mass cytometry method, as a method that combines flow cytometry and mass spectrometry, enables the simultaneous quantification of numerous cellular parameters (SA-β-GAL) at single-cell resolution (86). Also, among potential predictors of biological age could be included the degree of methylation of DNA, transcriptomic predictors, proteomic predictors, metabolomics-based predictors, and composite biomarker predictors (87).

Additional research is needed to confirm that glycans or some other compounds will meet necessary criteria to be the biomarkers of senescence. In the future, biomarker and therapeutic target candidates will be examined for a follow-up study, which will facilitate longitudinal monitoring of therapeutic interventions on senescence and aging.

Today, the bioinformatics, as an interdisciplinary field of science, helps to analyse and interpret biological data on aging and senescence, including studies of gene expression and comparative and pathway analyses (88-90). Computational biology of aging refers to a wide range of data, from demographic to genomic transcriptomic, proteomic and metabolomic studies (88). CSGene database has been developed for exploring cell senescence genes and to highlight the roles of cell senescence genes in the control of rRNA gene transcription (89).

Between 1997 and 2019, PubMed published about 363,000 articles on senescence and aging, and in the first four months of 2019, more than 10,000 articles. In this review, 90 articles have been selected to help us better understand the need to discover the hallmarks and biomarkers of senescence and aging. The knowledge of the mechanisms of senescence and the influence of senescence on aging of organism have evolved due to the development of numerous standard and sophisticated and laboratory methods. Senescence and aging can be observed from different aspects so that this topic can be observed in the context of research of mainly human fibroblasts, leukocytes, cell cultures and animal leukocytes and intestinal crypt enterocytes, dermal fibroblasts, hepatocytes, osteocytes, computational biology methods, the examination of factors involved in the normal pathways of acute and chronic senescence, diseases that can affect the process of senescence, processes that can repair senescence effects (5,7,10,11,16,21-23,27,43,50,78,81,88,89), etc. In order to successfully investigate these processes, it is necessary to find standardized biomarkers of senescence or the healthy aging of the organism (70). It is important to know the extent of determining a particular biomarker to prevent age-related assessment of the entire organism. Standardized biomarkers could also help in the monitoring of therapeutic interventions in the process of senescence, which is one of the goals of examining all aspects of senescence (11,21).

Instead of a conclusion

- The largest number of study of senescence and aging processes were made on cell cultures and animal models.
- The senescence seems to be a critical factor in both the normal aging process and pathologies associated with aging.
- There are currently no standardized biomarkers (“gold standard”) of cellular aging process or the healthy aging of the organism. Biomarkers described in literature do not meet all criteria of an ideal aging biomarker and actually represent various hallmarks of the aging process.
- Most biomarkers currently being examined as senescence or aging biomarkers are related to age-related illnesses rather than the process of healthy aging.
• As the effector mechanisms of senescence are neither necessarily specific to senescence nor present in all forms of senescence (the rate of senescence is not the same for all types of cells), the interpretation of existing biomarkers of senescence (for now the hallmarks or possible biomarkers) should be context dependent. Additionally, a combination of multiple biomarkers should be used.
• Detection of biomarkers, in particular their quantification and validation, are necessary for understanding the senescence processes (diagnostic biomarkers), monitoring of the rate of senescence (prognostic and predictive biomarkers) and the possible use of appropriate therapy intervention (pharmacodynamic biomarkers).
• The identification and selection of reliable biomarker(s), and the use of reproducible methods could help to better understanding of complex web of senescence and aging processes, but it will also open some new questions.
• Despite new findings at the cellular and molecular level the understanding the aging process is still limited.

Potential conflict of interest
None declared.

References
1. Strehler BL, ed. Understanding aging. In: Barnett YA, Barnett CR, eds. Aging Methods and Protocols. Methods in Molecular Medicine. Totowa: Humana Press Inc; 2000. p. 1-19. https://doi.org/10.1385/1-59259-070-5:1
2. Dyussenbayev A. The periods of human life. Glob J Human-Social Sci. 2017;17:32-6. https://doi.org/10.14738/assjr.46.2924
3. Strickland M, Yacoubi-Loueslati B, Bouhaouala-Zahar B, Pender SLF, Larbi A. Relationships between ion channels, mitochondrial functions and inflammation in human aging. Front Physiol. 2019;10:158. https://doi.org/10.3389/fphys.2019.00158
4. McHugh D, Gil J. Senescence and aging: Causes, consequences, and therapeutic avenues. J Cell Biol. 2018;217:65-77. https://doi.org/10.1083/jcb.201708092
5. Vicencio JM, Galluzzi L, Tajeddine N, Ortiz C, Criollo A, Tasdemir E, et al. Senescence, apoptosis or autophagy? When a damaged cell must decide its path – A mini-review. Gerontology. 2008;54:92-9. https://doi.org/10.1159/000129697
6. Faragher RGA, Mc Ardle A, Willows A, Ostler EL. Senescence in the aging process. F1000Res. 2017;6:1219. https://doi.org/10.12688/f1000research.10903.1
7. Yanagi S, Tsubouchi H, Miura A, Matsuo A, Matsumoto N, Nakazato M. The impacts of cellular senescence in elderly pneumonia and in age-related lung diseases that increase the risk of respiratory infections. Int J Mol Sci. 2017;18:E503. https://doi.org/10.3390/ijms18030503
8. Coppé JP, Desprez PY, Krüselica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol. 2010;5:99-118. https://doi.org/10.1146/annurev-pathol-121808-102144
9. Mrschtik M, Ryan KM. Lysosomal proteins in cell death and autophagy. FEBS J. 2015;282:1858-70. https://doi.org/10.1111/febs.13253
10. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med. 2015;21:1424-35. https://doi.org/10.1038/nm.4000
11. Watanabe S, Kawamoto S, Ohtani N, Harada E. Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases. Cancer Sci. 2017;108:563-9. https://doi.org/10.1111/cas.13184
12. Noren Hooten N, Evans MK. Techniques to induce and quantify cellular senescence. J Vis Exp. 2017;(123). 10.3791/55533. https://doi.org/10.3791/55533
13. Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs. Genes Dev. 2014;28:99-114. https://doi.org/10.1101/gad.235184.113
14. Velarde MC, Menon R. Positive and negative effects of cellular senescence during female reproductive aging and pregnancy. J Endocrinol. 2016;230:R59-R76. https://doi.org/10.1530/JOE-16-0018
15. Ogrodnik M, Salmonowicz H, Gladyshev VN. Integrating cellular senescence with the concept of damage accumulation in aging: Relevance for clearance of senescent cells. Aging Cell. 2019;18:e12841. https://doi.org/10.1111/acel.12841
16. van Deursen JM. The role of senescent cells in aging. Nature. 2014;509:439-46. https://doi.org/10.1038/nature13193
17. Jun Ji, Lau LF. Cellular senescence controls fibrosis in wound healing. Aging (Albany NY). 2010;2:627-31. https://doi.org/10.18632/aging.100201
Senescence and aging

18. Freund A, Orjala AV, Desprez P-Y, Campisi J. Inflammatory networks during cellular senescence: Causes and consequences. Trends Mol Med. 2010;16:238-46. https://doi.org/10.1016/j.molmed.2010.03.003

19. Özcan S, Alessio N, Acar MB, Mert E, Omerli F, Peluso G et al. Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. Aging (Albany NY). 2016;8:1316-29. https://doi.org/10.18632/aging.100971

20. Lu jámbio A. To clear, or not to clear (senescent cells)? That is the question. Bioessays. 2016;38 Suppl 1:S56-64. https://doi.org/10.1002/bies.201670910

21. Schmitt R. Senotherapy: growing old and staying young? Pflugers Arch. 2017;469:1051-9. https://doi.org/10.1007/s00424-017-1972-4

22. Rafi MA, Alavi A. Debate on human aging and lifespan. Bio-impacts. 2017;7:135-7. https://doi.org/10.15171/bi.2017.16

23. Korolchuk VI, Miwa S, Carroll B, von Zglinicki T. Mitochondria in cell senescence: Is mitochondy the weakest link? Ebiomedicine. 2017;21:7-13. https://doi.org/10.1016/j.ebom.2017.03.029

24. Childs BG, Baker DJ, Kirkland JL, Campisi J, van Deursen JM. Senescence and apoptosis: dueling or complementary cell fates? EMBO Rep. 2014;15:1139-53. https://doi.org/10.15252/embr.201439245

25. Arai Y, Martin-Ruiz CM, Takayama M, Abe Y, Takebayashi T, Koyasu S, et al. Inflammation, but not telomere length, predicts successful ageing at extreme old age: A longitudinal study of semi-supercentenarians. Ebiomedicine. 2015;2:1549-58. https://doi.org/10.1016/j.ebomi.2015.07.029

26. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;130:223-33. https://doi.org/10.1016/j.cell.2007.07.003

27. Barnes PJ. Mechanisms of development of morbidity in the elderly. Eur Respir J. 2015;45:790-806. https://doi.org/10.1183/09031936.00229714

28. Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Biochem J. 2001;353:411-6. https://doi.org/10.1042/bj3530411

29. Davalli P, Mitic T, Caporali A, ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med and Cell Longev. 2016;2016:3565127. https://doi.org/10.1155/2016/3565127

30. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med. 2018;54:287-93. https://doi.org/10.1016/j.ajme.2017.09.001

31. Ćepelak I, Dodig S. Glutathione and oxidative stress. Biochem Med (Zagreb) 2003;13:93-100.

32. Pole A, Dimri M, Dimri GP. Oxidative stress, cellular senescence and ageing. AIMS Mol Sci. 2016;3:300-24. https://doi.org/10.3934/molsci.2016.3.300

33. Passos JP, SaretziK G, Ahmed S, Nelson G, Richter T, Peters H, et al. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. PLoS Biol. 2007;5:e110. https://doi.org/10.1371/journal.pbiol.0050110

34. Ziegler DV, Wiley CD, Velarde MC. Mitochondrial effectors of cellular senescence: beyond the free radical theory of aging. Aging Cell. 2015;14:1-7. https://doi.org/10.1111/acel.12287

35. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-72. https://doi.org/10.2147/CIA.S158513

36. Siderakis M, Tarsounas M, Telomere regulation and function during meiosis. Chromosome Res. 2007;15:667-79. https://doi.org/10.1007/s10044-007-0711-9

37. Bernal A, Tusell L. Telomeres: Implications for cancer development. Int J Mol Sci. 2018;19:piiE294. https://doi.org/10.3390/ijms1910294

38. Sanders JL, Newman AB. Telomere length in epidemiology: A biomarker of aging, age-related disease, both, or neither? Epidemiol Rev. 2013;35:112-31. https://doi.org/10.1093/epirev/mxs008

39. Okuda K, Bardeguaz A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, et al. Telomere length in the newborn, Pediatr Res. 2002;52:377-81. https://doi.org/10.1203/00006450-20020900-00012

40. Bischoff C, Graaajkaer J, Petersen HC, Jeune B, Koefvraa S, et al. Telomere length among the elderly and oldest-old. Twin Res Hum Genet. 2005;8:425-32. https://doi.org/10.1373/twin.8.5.425

41. Kimura M, Hjelmborg JvB, Gardner JP, Rodriguez P, Ganesh V, Kimura M, et al. Telomere length in the newborn, Pediatr Res. 2002;52:377-81. https://doi.org/10.1203/00006450-20020900-00012

42. Arai Y, Martin-Ruiz CM, Takayama M, Abe Y, Takebayashi T, Koyasu S, et al. Inflammation, but not telomere length, predicts successful ageing at extreme old age: A longitudinal study of semi-supercentenarians. Ebiomedicine. 2015;2:1549-58. https://doi.org/10.1016/j.ebomi.2015.07.029

43. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;130:223-33. https://doi.org/10.1016/j.cell.2007.07.003

44. Galbiati A, Beauséjour C, d’Adda di Fagagna F. A novel skin cells tosenescent differentiation of senescent-associated heterochromatin foci. PLoS Genet. 2015;1:e1005024. https://doi.org/10.1371/journal.pgen.1005024

45. Arai Y, Martin-Ruiz CM, Takayama M, Abe Y, Takebayashi T, Koyasu S, et al. Inflammation, but not telomere length, predicts successful ageing at extreme old age: A longitudinal study of semi-supercentenarians. Ebiomedicine. 2015;2:1549-58. https://doi.org/10.1016/j.ebomi.2015.07.029

46. Arai Y, Martin-Ruiz CM, Takayama M, Abe Y, Takebayashi T, Koyasu S, et al. Inflammation, but not telomere length, predicts successful ageing at extreme old age: A longitudinal study of semi-supercentenarians. Ebiomedicine. 2015;2:1549-58. https://doi.org/10.1016/j.ebomi.2015.07.029

47. Cherednokarara A, del Pilar Sosa Ichdchel, Andrés Melendez J. Redox control of senescence and age-related disease. Redox Biology. 2017;11:91-102. https://doi.org/10.1016/j.redox.2016.11.005

48. Galbiati A, Beauséjour C, d’Adda di Fagagna F. A novel single-cell method provides direct evidence of persistent DNA damage in senescent cells and aged mammalian tissues. Aging Cell. 2017;16:422-7. https://doi.org/10.1111/acel.12573

49. Korolchuk VI, Miwa S, Carroll B, von Zglinicki T. Mitochondria in Cell Senescence: Is Mitophagy the Weakest Link? Ebiomedicine. 2017;21:7-13. https://doi.org/10.1016/j.ebomi.2017.03.020

50. Zhang R, Chen W, Adams PD. Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol. 2007;27:2343-58. https://doi.org/10.1128/MCB.02019-06

51. Bernadotte A, Mikhelson VM, Spivak IM. Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. Aging. 2016;8:3-11. https://doi.org/10.18632/aging.100871
48. Trindade LS, Aigaki T, Peixoto AA, Balduino A, Mânicada Cruz IB, Hedde JG. A novel classification system for evolutionar- 
ying aging theories. Front Genet. 2013;4:25. https://doi. 
org/10.3389/fgene.2013.00025

49. Sergeiv PV, Donsotova OA, Berezkin GV. Theories of aging: An 
ever-evolving field. Acta Naturae. 2015;7:9-18.

50. Jaul E, Barron J. Age-related diseases and clinical and public 
health implications for the 85 years old and over populati-
on, Front Public Health. 2017;5:50. https://doi.org/10.3389/
fpubh.2017.000335

51. Sharma G, Goodwin J. Effect of aging on respiratory system 
physiology and immunology. Clin Interv Aging. 2006;1:253-
60. https://doi.org/10.2147/cia.2006.1.3.253

52. Pizent A, Pavlovic M, Jurasovic J, Dodig S, Pašalić D, Mija-
gić R. Antioxidants, trace elements and metabolic syndro-
me in elderly subjects. J Nutr Health Aging. 2010;14:866-71. 
https://doi.org/10.1007/s12603-010-0139-1

53. Denic A, Glassock RJ. Rule AD. Structural and functional 
changes with the aging kidney. Adv Chronic Kidney Dis.  
2016;23:19-28. https://doi.org/10.1053/j.ackd.2015.08.004

54. Pulchinelli A Jr, Cury AJ Jr, Gimenes AC. Clinical laboratory 
findings in the elderly. J Bras Patol Med Lab. 2012;48:169-74. 
https://doi.org/10.1590/S1676-24442012000300004

55. Goldberg EL, Vishwa Dixa D. Drivers of age-related in-
flammation and strategies for healthspan extension. Immu-
no Rev. 2015;265:63-74. https://doi.org/10.1111/imr.12295

56. Minciuollo PL, Catalano A, Mandraffino G, Casciaro M, Cru-
citti A, Maltese G, et al. Inflammaging and anti-inflammag-
ing: The role of cytokines in extreme longevity. Arch 
Immunol Ther Exp (Warsz). 2016;64:111-26. https:// 
https://doi.org/10.1007/s00005-015-0377-3

57. Ventura MT, Casciaro M, Gangemi S, Buquicchio R. Immuno-
nsenescence in aging: between immune cells depletion and 
cytokines up-regulation. Clin Mol Allergy. 2017;15:21. 
PMDID:29259496. https://doi.org/10.1186/s12948-017-0077-0

58. Rea IM, Gibson DS, Mcgilligan V, McNerlan SE, Alexan-
der HD, Ross OA. Age and age-related diseases: Role of 
inflammation triggers and cytokines. Front имmu-
nol. 2018;9:article 586, p. 1-28. https://doi.org/10.3389/
fnmmu.2018.00586

59. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, 
et al. Inflammaging and anti-inflammaging: a systemic per-
spective on aging and longevity emerged from studies in 
humans. Mech Ageing Dev. 2007;128:92-105. https:// 
https://doi.org/10.1016/j.mad.2006.11.016

60. Lages CS, Suffia I, Velilla PA, Huang B, Warshaw G, Hildeman 
DA, et al. Functional regulatory T cells accumulate in aged 
hosts and promote chronic infectious disease reactivati-
on. J Immunol. 2008;181:1835-48. https://doi.org/10.4049/
immunol.181.3.1835

61. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer 
G. The hallmarks of aging. Cell. 2013;153:1194-217. https:// 
https://doi.org/10.1016/j.cell.2013.05.039

62. Aw D, Silva AB, Palmer DB. Immunoopause: emerging 
challenges for an aging population. Immunology. 
2007;120:435-46. https://doi.org/10.1111/j.1365-
2567.2007.02555.x

63. Busse PJ, Mathur SK. Age-related changes in immune func-
tion: effect on airway inflammation. J Allergy Clin Immunol. 
2010;126:690-9. https://doi.org/10.1016/j.jaci.2010.08.011

64. Poland GA, Ovsyanikova IG, Kennedy RB, Lambert N, Kirk-
land JL. A systems biology approach to the effect of aging, 
immunosenescence and vaccine response. Curr Op Immunol. 
2014;29:62-68. https://doi.org/10.1016/j. 
col.2014.04.005

65. Jagger A, Shimajima Y, Goronzy JJ, Weyand CM. T regu-
ulatory cells and immune aging process. Gerontology. 
2014;60:130-7. https://doi.org/10.1159/000355303

66. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Cau-
ses, consequences, and reversal of immune system aging. 
J Clin Invest. 2013;123:958-65. https://doi.org/10.1172/ 
JC64096

67. Engelfriet PM, Jansen EH, Picavet HS, Dollé ME. Biochemical 
markers of aging for longitudinal studies in humans. Epide-
miol Rev. 2013;35:132-51. https://doi.org/10.1093/epirev/
mxs011

68. Biomarkers Definition Working Group. Biomarkers and surro-
gate endpoints: preferred definitions and conceptual 
framework. Clin Pharmacol Ther. 2001;69:89-95. https:
https://doi.org/10.1067/mcp.2001.113989

69. WHO International Programme on Chemical Safety Bio-
markers in Risk Assessment: Validity and validation. Gene-
va, Switzerland 2001. Available at: http://www.who.int/iris/ 
handle/10665/42363. Accessed January 19th 2019.

70. Xia X, Chen W, McDermott J, Han J-D J. Molecular and phe-
notypic biomarkers of aging. F1000Res. 2017,6:860. https:
https://doi.org/10.12688/f1000research.10692.1

71. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Wor-
zella TJ, et al, eds. Cell viability assays. In: Sittampalam GS, 
Coussens NP, Brimacombe K, et al., eds. Assay Guide-
ly Manual [Internet]. Bethesda (MD): Eli Lilly & Company and 
the National Center for Advancing Translational Sciences;
2004. Available at: https://www.ncbi.nlm.nih.gov/books/
NBK144065/. Accessed January 20th 2019.

72. Zhang Z, Singh R, Aschner M. Methods for the detecti-
on of autophagy in mammalian cells. Curr Protoc Toxicol. 
2016;69:20.12.1-26. https://doi.org/10.1002/cptx.11

73. Biran A, Zada L, Abou Karam P, Vadić E, Roitman L, Ovdanya 
Y, et al. Quantitative identification of senescent cells in 
aging and disease. Aging Cell. 2017;16:661-71. https:
https://doi.org/10.1111/acel.12592

74. Wang L, Han X, Qu G, Su L, Zhao B, Miao J. A pH probe in-
hibits senescence in mesenchymal stem cells. Stem Cell Res 
Ther. 2018;9:343. 11 pages. https://doi.org/10.1186/s13287-
018-1081-0

75. Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, Cao X, 
et al. Measurement of telomere length by the Southern 
blot analysis of terminal restriction fragment lengths. Nat 
Protoc. 2010;5:1596–607. https://doi.org/10.1038/ 
nprot.2010.124

76. Montpetit AJ, Alhareeri AA, Montpetit M, Starkweather AR, 
Elmore LW, Filler K, et al. Telomere length: a review of met-
ods for measurement. Nurs Res. 2014;63:289-99. https:
https://doi.org/10.1179/NNR.0000000000000037
77. de Magalhães JP, Passos JF. Stress, cell senescence and organismal ageing. Mech Ageing Dev. 2018;170:2–9. https://doi.org/10.1016/j.mad.2017.07.001

78. Gao SG, Zeng C, Li LJ, Luo W, Zhang FJ, Tian J, et al. Correlation between senescence-associated beta-galactosidase expression in articular cartilage and disease severity of patients with knee osteoarthritis. Int J Rheum Dis. 2016;19:226-32. https://doi.org/10.1111/1756-185X.12096

79. Noppe G, Dekker P, de Koning-Treurniet C, Blom J, van Heemst D, Dirks RW. Rapid flow cytometric method for measuring senescence associated beta-galactosidase activity in human fibroblasts. Cytometry A. 2009;75:910-6. https://doi.org/10.1002/cyto.a.20796

80. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. J Gerontol A Biol Sci Med Sci. 2011;66:202-13. https://doi.org/10.1093/gerona/glq180

81. Aspinall R, ed. Aging of organs and systems. Dordrecht: Springer Science+Business Media; 2003. p.29-201. https://doi.org/10.1007/978-94-017-0673-5

82. Krštić J, Vučković M, Menni C, Klaric L, Keser T, Beceheli I, et al. Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci. 2014;69:779-89. https://doi.org/10.1093/gerona/glt190

83. Schosserer M, Grillari J, Breitenbach M. The dual role of cellular senescence in developing tumors and their response to cancer therapy. Front Oncol. 2017;7:278. p 1-13. https://doi.org/10.3389/fonc.2017.00278

84. Evangelou K, Lougiakis N, Rizou SV, Kotsinas A, Kletsas D, Muñoz-Espín D, et al. Robust universal biomarker assay to detect senescent cells in biological specimens. Aging Cell. 2017;16:192-7. https://doi.org/10.1111/acel.12545

85. Rizou SV, Evangelou K, Myrianthopoulos V, Mourouzis I, Havaki S, Athanasiou A, et al. A novel quantitative method for the detection of lipofuscin, the main by-product of cellular senescence, in fluids. Methods Mol Biol. 2019;1896:119-38. https://doi.org/10.1007/978-1-4939-8931-7_12

86. Lumba MA, Willis LM, Sanra S, Rana R, Schito L, Rey S, et al. A β-galactosidase probe for the detection of cellular senescence by mass cytometry. Org Biomol Chem. 2017;15:6388-92. https://doi.org/10.1039/C7OB01227F

87. Jylhävä J, Pedersen NI, Hagg S. Biological age predictors. EbioMedicine. 2017;21:29-36. https://doi.org/10.1016/j.ebiom.2017.03.046

88. Wieser D, Papatheodorou I, Ziehm M, Thornton JM. Computational biology for ageing. Phil Trans R Soc B 2011;366:51-63. https://doi.org/10.1098/rstb.2010.0286

89. Zhao M, Chen L, Qu H. CSGene: a literature-based database for cell senescence genes and its application to identify critical cell aging pathways and associated diseases. Cell Death Dis. 2016;7:e2053. https://doi.org/10.1038/cddis.2015.414

90. Hernandez-Segura A, de Jong TV, Melov S, Guryev V, Campisi J, Demaria M. Unmasking transcriptional heterogeneity in senescent cells. Curr Biol. 2017;27:2652-2660.e4.