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Review article

Evaluating the physiological reserves of older patients with cancer: The value of potential biomarkers of aging?

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ABSTRACT

Aging of an individual entails a progressive decline of functional reserves and loss of homeostasis that eventually lead to mortality. This process is highly individualized and is influenced by multiple genetic, epigenetic and environmental factors. This individualization and the diversity of factors influencing aging result in a significant heterogeneity among people with the same chronological age, representing a major challenge in daily oncology practice. Thus, many factors other than mere chronological age will contribute to treatment tolerance and outcome in the older patients with cancer. Clinical/comprehensive geriatric assessment can provide information on the general health status of individuals, but is far from perfect as a prognostic/predictive tool for individual patients. On the other hand, aging can also be assessed in terms of biological changes in certain tissues like the blood compartment which result from adaptive alterations due to past history of exposures, as well as intrinsic aging processes. There are major signs of 'aging' in lymphocytes (e.g. lymphocyte subset distribution, telomere length, p16INK4A expression), and also in (inflammatory) cytokine expression and gene expression patterns. These result from a combination of the above two processes, overlaying genetic predispositions which contribute significantly to the aging phenotype. These potential "aging biomarkers" might provide additional prognostic/predictive information supplementing clinical evaluation. The purpose of the current paper is to describe the most relevant potential "aging biomarkers" (markers that indicate the biological functional age of patients) which focus on the biological background, the (limited) available clinical data, and technical challenges.

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Chitinase
CRAMP
Aging genes

Despite their great potential interest, there is a need for much more (validated) clinical data before these biomarkers could be used in a routine clinical setting. This manuscript tries to provide a guideline on how these markers can be integrated in future research aimed at providing such data.

Contents

1. Introduction

The incidence of most malignant diseases increases with age.[1] Data from the Surveillance, Epidemiology, and End Results (SEER) database show that approximately 55% of all newly diagnosed cancer cases and 70% of cancer-related deaths occur in patients aged 65 years or older.[2] Median age at death for the major tumors common to both males and females (lung, colorectal, lymphoma, leukemia, pancreas, stomach, urinary bladder) ranges from 71 to 77 years.[2] Thus, as the world population ages, it is expected that the number of older patients with cancer will increase and therefore clinicians will be frequently confronted with older patients with cancer and treatment decisions in this population.[3]

Aging may contribute to carcinogenesis in two ways: first the passage of time simply leads to accumulations of cells with different molecular aberrations, eventually resulting in overt tumors; second, aging is associated with substantial alterations in internal homeostasis, especially in immune and endocrine systems that play a significant role in cancer control. Hence, aging is associated with numerous events at the molecular, cellular and physiological levels that increase susceptibility to carcinogens, promote carcinogenesis and decrease protective mechanisms.[4]

Cellular senescence is a fundamental cellular program that can be activated by different mechanisms. Deoxyribonucleic acid (DNA) damage is considered one of the most important triggers. If the amount of DNA damage after exposure to either endogenous or exogenous toxins is beyond the capacity of repair mechanisms, but fails to initiate apoptosis, the cell can activate a DNA damage response,[5] ultimately leading to permanent cell cycle arrest, i.e. senescence.[6,7] A second, in some ways related, trigger of cellular senescence is replicative exhaustion, i.e. cells can only undergo a finite number of divisions under some sort of genetic control, most often shortening of the repeats at the ends of the chromosomes (telomeres). When these reach a critical low number after numerous cell divisions, a signaling cascade is initiated (a DNA damage cascade — hence the similarities with the first mechanism above) and the cell is driven into a state of irreversible growth arrest.[5] Third mechanism is excessive exposure of the cell to oxidative stress can also lead to a similar response to that observed with replicative exhaustion, resulting in a permanently growth arrested senescent status.[8] Finally, another mechanism that can lead to activation of the senescence pathway and result in cellular senescence is the activation

of an oncogene, or the loss of function of a tumor suppression gene, a process termed oncogene-induced senescence.[9] Although the senescence response can be induced by diverse stimuli, the establishment and maintenance of the permanent growth arrest are commonly mediated by two major tumor suppressor pathways, p53 and p16INK4a-pRB. These seem to be more or less selectively engaged by different senescence stimuli.[6,7] The p53 pathway, with the cell cycle inhibitor p21 as an important downstream mediator,[10] primarily controls senescence induction in response to DNA damage. On the other hand, the p16INK4a-pRB involves the binding of Rb to the E2F family of transcription factors. It thereby inhibits downstream transcriptional pathways required for cell cycle entry.[11] The inhibitory effects of Rb on E2F are controlled through phosphorylation of Rb by cyclin/CDK complexes which, in turn, are inhibited by p16INK4a that also leads to permanent cell cycle arrest.[7] The relative importance of the two pathways in vivo is unknown, although a critical role for p16INK4a-pRB is generally accepted; p16INK4a has indeed been identified as a biomarker of senescence/aging in animal models.[12] Very recently, creating a new animal model for imaging of p16INK4a activation in vivo – the luciferase knock-in mouse $p16^{+/LUC}$ – Burd et al. [13] were able to show that activation of p16INK4a during physiological murine aging increased exponentially with chronological age throughout the body. Interestingly, there appeared to be significant inter-individual variability in the rate of luminescence change (reflecting p16INK4a activation) with aging in contemporaneously housed, syngeneic mice. However, correlation of p16INK4a activation levels with remaining life expectancy was not observed. Senescence acts to prevent further cell proliferation. It can be seen as a safeguard program that limits the growth capacity of a potentially harmful dividing cell and thus could act as a protection mechanism against cancer. On the other hand, it is also hypothesized to be a driving force in aging: accumulation in the organism of senescent cells, which lack regeneration capacity, may result in failure of organ homeostasis and function and, consequently, in tissue aging.[7,12,14] Furthermore, senescent cells have themselves a harmful pro-inflammatory phenotype, and are more sensitive to carcinogenesis.[15]

Aging of an organism (in contrast to aging of a cell) is a highly individualized process, which is seen as a progressive decline of functional reserves and loss of homeostasis that eventually lead to mortality. As such, it is influenced by multiple genetic, epigenetic and environmental factors.[16,17] This individualization and the diversity of factors influencing aging result in a significant heterogeneity among people with the same chronological age. Some present with significant functional impairment and several comorbidities while others do not have significant dependence and major comorbidities.[18,19] It would be reasonable to assume that the variation in general health status seen in older people without cancer will also be observed in older people with cancer, possibly in a more exaggerated manner due to the presence of the tumor, as well as possible pro-aging effects of many cancer therapies. Individual variations in health status represent a major challenge clinicians face in daily oncology practice. In order to plan individual therapeutic strategies, oncologists need to have reliable information about "how old" the patient actually is. Practically, a clinician wants to know whether the patient's general health status and comorbidities will determine prognosis or whether the newly-diagnosed cancer will limit life expectancy.[20] In other words, is the patient going to die of the cancer or with the cancer? This is particularly important when treatments are being considered in the adjuvant setting, where the maximal benefits of treatment on reducing the risks of recurrence may only be realized many years later. The clinician also wants to know whether the patient will be able to tolerate conventional anti-cancer treatments (surgery, radiotherapy, chemotherapy, or multimodal treatment) without experiencing severe toxicities, deterioration of health-related quality of life, and without the occurrence of treatment-related mortality.[21] These two factors: assessing longevity and assessing frailty, while related, are not the same, and different biomarkers may predict one and not the other. These factors are all associated in some way with age, but chronological age is an inaccurate indicator of such susceptibility. Clinical/comprehensive geriatric assessment has been established as a way to deal with this heterogeneity, and allows us to obtain a better view of the global health status including functional, nutritional, social and cognitive status, and the patient's non-oncological health condition.[21–27] Clinical tools such as the Lee score,[20] or other tools available online at www.eprognosis.org provide some prognostic and to a lesser degree predictive information. These tools have been described in previous publications and therefore won't be presented here, but it should be acknowledged that their predictive value at the individual level remains at most moderate and is certainly far from perfect.

On the other hand, there are major signs of 'aging' in peripheral blood that can be quantified and have been shown to correlate with functional age and outcome in non-oncological patients. These potential "aging biomarkers" might provide additional prognostic/predictive information besides clinical evaluation. Biological markers of aging according to Falandry et al. need to be assessed on easily accessible samples, independent of specific pathologic conditions and validated through various, widely-accepted end-points such as overall survival and development of frailty.[15] The purpose of the current paper is to describe potential "biomarkers of aging" including biological background, clinical data and technical issues. It must be emphasized, however, that valid and reliable biomarkers of aging have not yet been identified and that none of the candidate markers discussed here have reached a sufficient level of evidence-based acceptance to allow their use in routine clinical practice. Further research is needed, and this manuscript aims to provide a guideline on how these markers can be integrated therein. The potential biomarkers of aging that will be discussed are summarized in Table 1.

2. Circulating Inflammatory Markers

A role for inflammation in the process of aging and age-related disease has been clearly established in several large epidemiological studies of older adults. Although acute inflammatory responses are closely regulated in the elderly, a low-level elevation of inflammatory markers is commonly observed and is associated with several chronic conditions of aging such as physical and cognitive decline, cardiovascular diseases and diabetes, or cancer.[28] While antigen-directed/adaptive immune responses usually decline with aging, general/non-specific

Table 1 – Potential biomarkers of aging.

Biomarker	
Inflammatory markers	IL-6 associated with functional disability[39] and "frailty" phenotype[40] IL-1, IL-6 and TNF-a are predictive for mortality[44] Activation of inflammatory pathways is associated with mortality and decline in function[46] Association observed between circulating levels of inflammatory markers (IL-6, TNF-α and CRP) and cancer mortality[54]
Telomere length/telomerase activity	Telomere length associated with years of healthy living,[63] with cancer prognosis[143] and human longevity[83]
Genetic predisposition for longevity	Inflammation-related genes[89] Genes involved in lipid metabolism[94] DNA repair genes[99] Cell cycle regulators/tumor suppressors[103] Genes involved in insulin/IGF-1 signaling[105]
Gene expression of aging related genes in peripheral blood mononuclear cell	Changes in the expression of age-related genes can be considered as a biomarker of aging[134]
Immunosenescence	The "immune risk profile" comprising an inverted CD4:CD8 ratio, accumulation of $CD8^+$ $CD28^-$ late-differentiated T cells, poor proliferative capacity, few B cells and CMV-seropositivity, is associated with significantly increased 2-, 4- and 6-year mortality in very elderly people[119,120] Expression of CD95 on lymphocytes can be predictive of successful aging[123] Impaired innate immunity as measured by low production capacity of pro- and anti-inflammatory cytokines is predictive of frailty in elderly and associated with a two-fold overall mortality risk[124]
Lymphocyte senescence: $p16^{INK4a}$ expression in T lymphocytes	Expression of $p16^{INK4a}$ increases with physical inactivity and exposure to mutagens such as tobacco and exponentially with chronological age, with an average 10-fold increase between the age of 20 and 80[132]
Plasma microRNA expression profile	Several miRNAs show aging-associated expression changes[142]

IL = interleukin, TNF = tumor necrosis factor, CRP = C-reactive protein.

inflammation seems often to increase in the elderly. The term inflammageing refers to a low-grade pro-inflammatory phenotype which accompanies aging in mammals.[15] Several reports have described an increase in pro-inflammatory cytokines (mainly IL-1, IL-6, and TNF-α), chemokines (IL-8, RANTES, MCP-1) and C-reactive protein (CRP), with increasing age even in healthy older persons without apparent presence of acute inflammation, while at the same time there is a decrease in anti-inflammatory mediators such as IL-10[28–30] and circulating levels of pro-inflammatory markers, such as IL-17, were significantly lower in elderly healthy individuals compared to younger ones.[31] With increasing comorbidity (increase in Cumulative Illness Rating Scale for Geriatrics [CIRS-G] score) in older adults, there is a proportional decrease in immune response (e.g. decrease in IL-12 production). However, there are disparate data showing age to be positively correlated with circulating levels not only of IL-12p70, IL-1β, TNFα, and IL-6, but also of IL-10.[31] Although the reasons for these disparities and for the heterogeneity seen in different studies and in different cohorts are not fully clear, numerous mechanisms differing between populations and studies have been proposed. These include cumulative oxidative damage that promotes inflammatory responses, declining levels of sex hormones after menopause and andropause,[28] increasing visceral adiposity, and chronic immune stimulation by persistent irritants especially latent viral infections, most particularly cytomegalovirus.[32] An alternative mechanism that was hypothesized by Campisi et al. is the systemic repercussion of the local microenvironment changes induced by senescent (and ultimately by neoplastic) cells.[33]

It was proposed that senescent or damaged cells acquire a 'senescence-associated secretory profile' or SASP, which can reinforce the proliferative arrest of these potentially harmful cells. However, it is also known that this secretory profile influences the microenvironment around these cells in such a way that it can promote malignant phenotypes in cells nearby (paracrine signaling). Moreover, the SASP has been shown to efficiently trigger the influx of several types of immune cells necessary for antitumor immunity (mostly through pro-inflammatory cytokines like IL-6 and IL-8, the protease inhibitor plasminogen activator inhibitor-1 (PAI-1) and the pleiotropic protein insulin-like growth factor binding protein-7 [IGFBP-7]) and therefore could be critical to tumor surveillance.[33,34] Clearance of the damaged cells by these infiltrating immune cells results in tumor surveillance, which seems to make the SASP a predominantly anti-tumor mechanism.[34,35] However, it has also been described that this secretory profile influences the microenvironment around these cells in such a way that it can paradoxically promote malignant phenotypes in cells nearby by paracrine signaling. Senescent fibroblasts seem able to alter the behavior of premalignant epithelial cells to a fully invasive phenotype[36] and stimulate their growth in vitro and in vivo (xenograft models).[37] Whereas the maintenance of growth arrest and promotion of tumor surveillance by immune influx seems to result from the induction of the SASP in the (pre)neoplastic cells themselves, the data on paradoxical tumor promotion mostly come from research focusing on the stromal cells surrounding the potentially neoplastic epithelial cells. Recently, in vivo imaging of the senescence process in a model of normal physiological aging[13] showed no correlation of the level of p16INK4A activation with the risk of death from cancer, which seems to contradict the fact that the accumulation of senescent cells in the body (assuming the changes in microenvironment due to the SASP) would predispose to cancer formation. Because it was also shown in

vitro that senescence mediated by activation of p16INK4A might not always be paralleled by the induction of the SASP,[38] it would be interesting to know if these p16INK4a-positive cells indeed had acquired the senescence-associated secretory profile. We want to emphasize the need to distinguish between local effects within the tissue, which may be beneficial, and systemic effects of the same cytokines, which may be inappropriate and eventually deleterious.

In community-dwelling elderly there is a clear association between IL-6 levels and functional disability[39] and a "frailty" phenotype.[40] In longitudinal studies of the very elderly (>85 yr), plasma IL-6 levels, together with cognitive impairment, formed a cluster independently correlating with 2-, 4- and 6-year mortality on follow-up.[32] Interestingly, peripheral cytokine levels are often elevated prior to cognitive decline,[41] dementia[42] and loss of physical performance.[43] Furthermore, markers of inflammation are considered as a predictive tool for mortality.[44,45] Evaluation of the Duke Established Populations for Epidemiologic Studies of the Elderly database (n = 2569 subjects; age > 71 years) demonstrated that activation of the coagulation and inflammatory pathways is associated with mortality and decline in function. The relative risk of mortality was 1.28 (95% confidence interval [CI]: 0.98 to 1.69; p-value = 0.06) for those with only interleukin-6 levels in the highest quartile, 1.53 (95% CI: 1.18 to 1.97; p-value = 0.001) for subjects with only D-dimer levels in the highest quartile, and 2.00 (95% CI: 1.53 to 2.62; p-value = 0.0001) for those with levels of both in the highest quartile, as compared with those who were not in either of the highest quartiles.[46] Data from the Newcastle 85+ Study (n = 845 subjects, age ≥ 85 years) confirmed the association between frailty and IL-6, TNF-alpha, CRP and neutrophil count in the very old.[47] It should be noted, however, that the association between elevated inflammatory biomarker levels and age-related phenotypes (cognitive decline, atherosclerosis, etc.) does not necessarily reflect a causal relationship. For instance, genome-wide association studies have revealed polymorphisms that are strongly associated with plasma levels of CRP, while no persuasive correlations could be established between these genetic variants and specific aging phenotypes in large-scale studies.[48,49] Therefore, inflammatory mediators may epiphenomenologically show increased expression with advancing age without being causal determinants of the aging process.

The role of inflammation in cancer as a causal factor and as a prognostic/predictive indicator has been widely studied. However, a systematic review by Heikkila et al. failed to identify any potential role for IL-6 in diagnosing or predicting cancer.[50] A second review by the same group found no strong evidence for higher CRP levels as a causative factor for cancer.[51] However it should be noted that both these reviews included only a small number of prospective studies and this weakens the power of the observations. Elevated CRP was a prognostic factor for poorer survival in patients with localized renal cell carcinoma[52] and high D-dimer was associated with poorer survival in patients with metastatic colorectal cancer.[53] A report from the Health Aging and Body Composition study (n = 2348 adults, 70–79 years of age) evaluating incident cancer cases (n = 296) observed an association between circulating levels of three inflammatory markers (IL-6, TNF-α and CRP) and cancer mortality [hazard ratios (HR) of 1.63 (95% CI: 1.19–2.23), 1.64 (95% CI: 1.20–2.24) and 1.82 (95% CI: 1.14–2.92) for IL-6, CRP and TNF-α, respectively].[54] Similarly the CLUE II study identified a positive association between high levels of CRP and colorectal cancer.[55] However, an important issue that limits the clinical use of inflammatory factors as potential "aging biomarkers" is the fact that their blood levels are dependent on inflammatory reactions caused by underlying health conditions, such as obesity. The timing of testing may also represent an important confounding factor, e.g. assessing inflammatory factors in patients post-operatively after cancer resection to judge longevity/fitness for adjuvant chemotherapy, might result in misleading conclusions because recent surgery itself mostly likely increases IL-6/CRP levels. Therefore, a solution could be to standardize the time in the disease pathway when blood samples are taken. It is demonstrated that the presence of short telomeres (a hallmark feature of aging) was associated with increased expression of different cytokines and inflammatory markers.[15,56] Also the timing of testing is important e.g. assessing inflammatory factors in patients post-operatively after cancer resection to judge longevity/fitness for adjuvant chemotherapy, might result in misleading conclusions because recent surgery might well increase IL-6/CRP levels. Therefore, a solution could be to standardize the time in the disease pathway when blood samples are taken.

3. Telomere Dynamics and Mean Leukocyte Telomere Length

Telomeres are DNA–protein complexes of repetitive DNA sequences and telomere binding proteins.[57] Their major function is to cap chromosomal ends and thereby preserve chromosomal stability.[58] When cells divide, the telomere is not fully replicated, leading to telomere shortening with every replication. Therefore, with each round of cell division, telomeres gradually shorten and eventually reach a critical value, leading to genomic instability, cessation of proliferation and replicative senescence.[59] Germ cells and stem cell populations express telomerase, a reverse transcriptase that elongates telomeres.[60] However, in somatic cells, telomerase activity is absent or is insufficient to prevent telomere shortening associated with cell division.[61] The gradual loss of telomere DNA thus implies that in normal tissues, the number of cell divisions is limited; this is commonly referred to as 'the mitotic clock'. The rate of telomere loss may also be modified by parameters such as systemic exposure to smoking or obesity that contribute to oxidative stress and age-related diseases, whereas a healthy life style might promote a more stable telomere length.[62] Therefore, telomere length can serve as an indicator of a cell's biological rather than chronological "age". In contrast, cancer cell often acquires the capacity to elongate telomeres again leading to immortality.

In a population-based cohort study with 3075 healthy, well-functioning men and women aged 70–79 years[63] leukocyte telomere length, although not associated with overall survival (HR 1.0; 95% CI 0.9–1.1) or death from any specific underlying cause including cancer, was positively associated with more years of healthy living. According to the authors, these findings suggest that although such crude assays of average telomere length may not yield strong biomarkers of survival in older individuals, they may be informative for healthy aging. Several other studies have found an association between telomeres and

Table 2 – Association between telomere length and "biological age" of older patients.

Study	N	Age	Telomere length and "biological age" association
Risques et al.[144]	624	>65	Short TL associated with disability and functional status as assessed by ADL.
Yaffe et al.[145]	2734	Mean age: 74	Shorter TL associated with worse cognitive function
Epel et al.[146]	236	70–79	TL shortening was associated with higher cardiovascular mortality
Farzaneh-Far et al.[147]	780	>65	Reduced TL associated with all caused mortality in patients with coronary artery disease.
Cawthon et al.[148]	143	>60	Individuals with shorter TL had 3.18-fold higher mortality rate.
Atzmon et al.[149]	354	NA	Case control study. Longer TM associated with protection form age-related diseases.

TL: telomere length; TM: telomere.

"biological age" of older patients (Table 2). Telomere length has been associated with several age-related diseases such as cardiovascular disease,[64] heart failure,[65] osteoporosis[66] and obesity.[67] Thus, telomere length assays should be further evaluated concerning their usefulness to estimate a patient's global health status and treatment tolerance.

Telomere length has also gained considerable interest as a potential biomarker of cancer risk. Several retrospective studies have found a strong association between shorter telomere length and cancer risk, including bladder,[68] breast,[69] gastroesophageal[70,71] and lung cancers.[72] Short telomere length has also been associated with negative prognosis in patients with colorectal cancer,[73] soft-tissue tumors,[74] breast cancer[75] and lung cancer.[76] Finally, a longitudinal population-based study demonstrated a statistically significant correlation between short telomere length and higher cancer incidence and mortality.[77] On the other hand, many prospective studies failed to demonstrate any significant association between short telomere length in leukocytes and increased cancer risk.[78] Additionally, other studies failed to demonstrate any association between telomere length and morbidity or mortality in the older and especially in the oldest old.[47,79,80] A potential explanation of these discordant results could be the fact that telomere length might also be controlled by genetic factors that result in differences between individuals[81] or different ethnical groups.[82] Furthermore, telomere length might be determined by genetic variants in SNPs (single nucleotide polymorphisms) and/or epigenetic modifications.[83,84] It should be acknowledged that telomere length measurement techniques have intrinsic technical limitations, and that large inter-individual differences exist of which the biological meaning is not well known. Measurement of telomere length can be performed in different ways (mean telomere length by PCR or TRF, shortest telomere by FISH) each with their own advantages and disadvantages.[85] Although telomere biology is extremely interesting, the (prognostic/predictive) value of telomere length in an individual patient is not yet established. A major problem remaining to be overcome in this respect is that most techniques measure the average telomere length of all chromosomes in the cell, whereas the shortest may be crucial for triggering e.g. replicative senescence, regardless of the overall value.

3.1. *Telomere Dysfunction Biomarkers: Chitinase and CRAMP*

Recent data also show that replicative senescence is associated with the secretion of several soluble factors in addition to cytokines/chemokines, e.g. cathelin-related antimicrobial peptide (CRAMP), stathmin, EF-1α, and chitotriosidase, a member of the chitinase family.[86] These factors were secreted by bone marrow cells with short, dysfunctional telomeres, but not from cells with long telomere reserves. They could also be detected in the serum/plasma of patients, and were found to be higher in the elderly, certainly when comorbidity was present.[86] Expression levels of these markers have adequate discriminatory ability to distinguish older people from younger ones as well as fit older individuals from elderly patients with Alzheimer's disease.[87]

4. Genetic Predisposition for Longevity

4.1. *Genes Involved in Inflammation*

Many studies have shown that variations in cytokine genes may affect the regulation of inflammation and might therefore play a role in determining human longevity. The inflammatory cytokine interleukin-1 (IL-1) is potentially involved in cognitive deterioration and Alzheimer-related neurodegenerative processes; genetic variants in the IL-1 gene cluster were indeed shown to be associated with cognitive performance in the elderly[88]. Genotype and allele frequencies of the −1082G/A polymorphism in the promoter region of the anti-inflammatory cytokine IL-10 gene were significantly associated with longevity in men (p-value < 0.05) but not in women.[89] Discordant results were obtained concerning the relationship between the IL-6 promoter (−174G/C) SNP, which is an important genetic factor determining IL-6 production levels, and longevity.[90] No data exist regarding the potential prognostic or predictive role of these genes in elderly patients with cancer.

4.2. *Genes Involved in Lipid Metabolism*

Apolipoproteins (Apos) belong to a group of proteins that play a key role in cholesterol and lipid metabolism.[91] Abnormal apolipoprotein levels have been observed in numerous age-related diseases, including Alzheimer's disease,[92] cognitive decline[93] and coronary heart disease.[91]

Apolipoprotein E (ApoE) polymorphism has been extensively studied as a biomarker of frailty and possession of the ApoE4 allele was found to be associated with frailty development in the elderly.[94] The same allele has been associated with increased relative risk of death. A recent genome-wide

association study (GWAS) even identified ApoE as the major locus determining survival into old age.[95] Another genome screen suggested that haplotypes in the gene encoding low-density lipoprotein receptor-related protein 1B (LRP1B) are significantly protective for successful aging without cognitive decline.[96] Paraoxonase 1 (PON1), a major anti-atherosclerotic component of high-density lipoprotein (HDL) that functions as an antioxidant by preventing the oxidation of LDL, has also been described as a longevity gene. In fact, it is one of the most studied genes regarding cardiovascular risk, oxidative stress and inflammation. It was shown that gene variants at codon 192 impact on the probability of reaching extreme ages.[97] No data exist specifically for patients with cancer.

4.3. *Genes Involved in DNA Repair*

DNA damage that is not repaired will lead to apoptosis or cellular senescence. Rare genetic syndromes which impede DNA repair are known to result in premature aging-like syndromes and/or predisposition to cancer (for example Cockayne syndrome, tricothiodystrophy and ataxia telangiectasia, but also Xeroderma Pigmentosum). So not surprisingly, DNA repair genes seem crucial for healthy aging.[98]

Several DNA base excision repair gene polymorphisms have been found to modulate human cognitive performance and decline during normal life span.[99] Also, Nebel et al. found that a functional exonuclease 1 (EXO1) promoter variant is associated with prolonged life expectancy in centenarians.[100] Furthermore, a functional SNP in the promoter of the 'ataxia telangiectasia mutated' (ATM) gene which encodes a serine/threonine kinase that is recruited by DNA double strand breaks and activates key proteins of the DNA damage response, was found to be associated with longevity.[101]

4.4. *Cell Cycle Regulators/Tumor Suppressors*

Gravina et al. showed that the rare alleles of two functional exon-derived SNPs in the p21/CDKN1A gene were significantly underrepresented among centenarians and, therefore, may be potentially detrimental to longevity.[102] Moreover, a common variant of the p16INK4a genetic region appeared to be associated with physical function in older people.[103] Furthermore, a genomic Arg72Pro substitution in the p53 protein with important influence on cell death via apoptosis was associated with increased longevity and improved survival after the development of cancer, but not with decreased risk of cancer in a large cohort of 9219 participants aged 20–95 years. Median overall survival increased by 3 years for Pro/Pro versus Arg/Arg homozygotes.[104]

4.5. *Genes Involved in Insulin/IGF-1 Signaling*

Numerous studies have shown that aging is hormonally influenced by an evolutionarily conserved insulin/IGF-1 signaling (IIS) pathway.[105] Levels of insulin-like growth factor 1 (IGF-1) decline with advancing age, and there is some data supporting a crucial role of this molecular mediator in the decline of functional reserve.[96] In the WHAS I study, however, low IGF-1 and high IL-6 levels were associated with a marked increase in walking limitation and an increase in 5-year mortality; (46% vs 23% for patients with high IGF-1 and low IL-6 levels).[97] Caloric restriction has been proven to be successful in prolonging life span at least in rodents.[106]

A prospective follow-up study investigating the relationship between IIS, body height and longevity in humans revealed that genetic variants resulting in lower IIS activity, particularly selected variants in the IIS components GH1, IGF1 and IRS1, are significantly associated with lower body height and improved old age survival in females.[107] Moreover, a recent GWAS study also highlighted the relevance of the IIS signaling pathway for human longevity.[83]

FOXO3A belongs to the forkhead family of transcription factors. It is involved in cell cycle arrest, oxidative stress resistance and apoptosis induction and is therefore known as a tumor suppressor. Its transcriptional activity is inhibited by insulin and/or IGF1 signaling through direct phosphorylation by protein kinase AKT/PKB and relocation from the nucleus to the cytosol. Genetic variation within the FOXO3a gene was reported to be strongly associated with longevity[108–110]. Moreover, genetic variants of the gene seem also to be associated with morbidity and/or mortality risk at older age.[111]

4.6. *Genome Wide Association Studies*

Although candidate SNP studies provide the means for identifying polymorphisms associated with the onset of complex phenotypes associated with aging, it should be emphasized that the majority of the studies discussed above are rather small and, except for the ApoE genotype, the observed associations between SNPs and specific aging phenotypes are not very strong. With the advent of population-based genome wide association studies (GWAS), applying SNP arrays to identify genes that may promote complex phenotypes, it has become possible to associate age-related phenotypes with human genetics on a large scale and in an non-biased manner.[112] GWAS have identified a large number of susceptibility SNPs associated with many important human diseases, including those prominent in aging. Jeck et al. performed a meta-analysis of 372 GWAS that identified 1775 susceptibility SNPs for 105 unique diseases and used these SNPs to create a genomic landscape of disease susceptibility.[113] To better study chromosomal loci and candidate genes associated with age-associated diseases, the authors summed the frequency of disease-associated SNPs in 200 kb 'bins' covering the whole genome. They identified 10 bins that were significantly enriched for susceptibility to multiple diseases. These comprised two highly significant peaks that mapped to the Major Histocompatibility (MHC) locus on 6p21 and the *INK4/ARF* (*CDKN2a/b*) tumor suppressor locus on 9p21.3. Interestingly, all the genes contained within these 10 enriched bins were linked to either inflammation pathway or cellular senescence pathway. Among these genes was also the telomerase subunit TERT, which is involved in cellular senescence by modulating telomere length. Moreover, SNPs near regulators of senescence were particularly associated with diseases of aging (e.g., cancer, atherosclerosis, type 2 diabetes, glaucoma), suggesting a causal link between cellular senescence and these age-associated pathologies.[113] Because they seem to be intrinsic

to the aging process, p16INK4a and telomere length may be of great value as aging biomarkers.

5. Immunosenescence

5.1. *Phenotypic and Functional Changes in Immune Cell Subsets*

Aging is associated with alterations of the immune system, which are thought to increase the susceptibility of older people to infectious diseases and possibly to cancer.[114] These alterations include those resulting from the early developmental process of thymic involution,[115] changes in the number, distribution, and activity of T- and B-lymphocytes,[116] reduced availability of $CD4^+$ and $CD8^+$ T-cells[117] and reduced production of naïve B-cells in the bone marrow.[118]

Recent cumulative evidence points towards age-associated changes in the cellular components of the innate immune system, including natural killer (NK) cells, phagocytes and dendritic cells.[119] Within the T-lymphocyte compartment, inversion of the CD4:CD8 ratio appears to be significantly associated with increased mortality at 2-, 4- and 6-year follow-ups in the very-elderly (>85 years) and has been documented as part of an "immune risk profile (IRP)".[119,120] The cluster of parameters constituting the IRP also includes latent cytomegalovirus (CMV) infection, which likely contributes to immune exhaustion through chronic antigen load and massive expansion of CMV-specific $CD8^+CD28^-$ effector/memory T cell clones, thereby decreasing the capacity of directed immune response against new pathogens. Many investigators have reported that peripheral blood mononuclear cells from the elderly generally contain decreased percentages of naïve $CD8^+$ cells and an increased proportion of late-stage differentiated $CD8^+$ cells with reduced proliferative capacity.[121] An age-associated increase in peripheral blood $CD4^+CD25^{high}$ regulatory T cells (T_{reg}), capable of decreasing cytotoxic activity of $CD8^+$ T and NK cells and reducing IL-2 production, has also been reported.[122] A study by Potestio et al. additionally found that expression of CD95 on the different subsets of lymphocytes can be predictive of successful aging and can be used as a marker of aging,[123] while a study from van der Biggelaar et al. demonstrated that impaired innate immunity as measured by low production capacity of pro- and anti-inflammatory cytokines is predictive of frailty in the elderly and is associated with a two-fold overall mortality risk.[124] The Newcastle 85+ study (a population-based study; n = 845, ≥85 year-old subjects) demonstrated that lymphopenia is a marker of frailty.[47] In oncology, baseline lymphopenia is associated with higher risk for hematological toxicity after chemotherapy[125,126] and higher risk of death after brain radiotherapy.[127] Lymphopenia is also found to be an independent prognostic factor for overall and progression-free survival in several malignancies and is part of the Palliative Prognostic Score.[128,129] As already discussed, dysfunctional telomeres are typical in aged cells and induce the production of inflammatory signals.[15] These signals are believed to have a negative impact on lymphopoiesis; this may underlie the connection between immunosenescence and the "inflammatory" profile observed in older individuals as discussed above.

5.2. *Lymphocyte Senescence: $p16^{INK4a}$ Expression in T Lymphocytes*

Lymphocyte senescence, and thus aging of the immune system, is reflected by increased mRNA expression of the cell cycle regulator $p16^{INK4a}$.[130] The *$p16^{INK4a}$* gene, a general regulator of senescence, is part of the *INK4a/ARF* locus, which encodes two protein products: the $p16^{INK4a}$ protein, an inhibitor of cyclin-dependent kinase 4/6 and the $p14^{ARF}$ protein, a potent regulator of p53 stability. Both proteins play non-redundant roles in mediating cellular senescence and in suppressing malignancies. In healthy humans, $p16^{INK4a}$ expression in peripheral blood T lymphocytes increases markedly with physical inactivity and exposure to mutagens such as tobacco.[131] Also, T lymphocyte expression of $p16^{INK4a}$ increases exponentially with chronological age, with an average 10-fold increase between the ages of 20 and 80.[132] A recent study[133] investigated the effect of adjuvant chemotherapy on T cell expression of $p16^{INK4a}$ in older women with early stage breast cancer. In patients over the age of 65, chemotherapy seems to be associated with a more than one decade-equivalent increase in expression of $p16^{INK4a}$. Log_2 [$p16^{INK4a}$] mRNA expression in patients without chemotherapy (n = 42) and with chemotherapy (n = 31) was 7.52 ± 0.15 and 8.12 ± 0.14, respectively (p-value = 0.009). Although preliminary, these data suggest that chemotherapy might possibly contribute, either directly or indirectly, to the precipitation of some aspects of the process of aging.

5.3. *Expression of Immune-related Genes in Peripheral Blood Mononuclear Cells*

A recent study by Vo et al. surveyed the differential expression of 148 putatively age-related genes in two groups of healthy young to middle-aged (35.0 ± 6.5 years old) versus old people (82.5 ± 6.8 years old).[134] This study provided a list of 16 differentially abundant transcripts in healthy old subjects. These differences encompassed diverse genes involved in adaptive immunity, such as CD28, CD69, LCK (decreased in old) and the antigen processing and presentation genes CD86, cathepsin D, H and S (increased in old), which thus could all be considered as easily accessible biomarkers of aging. Imputed changes in the abundance of these transcripts might reflect some of the functional alterations observed in the immune system, including the low-grade pro-inflammatory status observed in old persons and hypo-responsiveness of T-cells together with an increase in antigen presentation potential.[134]

6. Oxidative Stress Markers

According to the free radical theory of aging, *oxidative stress* increases with age, leading to accumulation of oxidation products of lipids, nucleic acids, proteins, sugars and sterols that exert deleterious effects and ultimately cause cellular dysfunction.[126] The most recent studies support the idea that oxidative stress is a

significant marker of senescence.[127] While production of reactive oxygen species (ROS) is a consequence of basal cellular respiration, increased ROS production is associated with several pathological conditions, including cancer. ROS accumulation over time is assumed to induce somatic mutations, and oxidative stress is directly associated with the development of prostate and chronic inflammatory bowel disease-related colorectal cancers.[128–130] Circulating markers of oxidative stress that can be measured include 8-iso-Prostaglandin F2[131–133] and 8-hydroxy-2′-deoxy-guanosine.[134,135] However, very few clinical data are available at present; in fact some reports call into question or even explicitly reject the hypothesis that oxidative damage plays a role in the process of aging.[135]

6.1. *MicroRNA Expression Profiles*

MicroRNAs (miRNAS) are small non-coding RNAs that repress or inhibit gene expression by targeting messenger RNA (mRNA).[136] MiRNAs are involved in the regulation of a variety of physiological processes such as cellular development, differentiation, proliferation, apoptosis, and metabolism,[137] while aberrant miRNA expression has been implicated in many human disorders including cardiovascular disease, autoimmune diseases and cancer.[138] Therefore, miRNA expression profiling might serve as a potential diagnostic and prognostic biomarker for these conditions.[139,140] Recent research has highlighted an important role of miRNAs in the aging process. Analysis of the miRNA expression profiles in PBMC from elderly versus young individuals revealed significant age-associated down-regulation of miR-24, miR-103, miR-107, miR-128, miR-130a, miR-155, miR-221, miR-496 and miR-1538.[141] Furthermore, a very recent genome-wide study[142] using a microarray technique comprising 863 miRNAs compared the blood expression profiles of 15 centenarians and nonagenarians (mean age 96.4 years) with those of 55 younger individuals (mean age 45.9 years). Eighty miRNAs showed aging-associated expression changes, with 16 miRNAs being up-regulated and 64 down-regulated in the old relative to the younger subjects

7. Measuring Biomarkers of Aging: Practical Aspects of Biobanking

This section will give some suggestions as to how aging biomarker assessments can be incorporated into clinical studies, by providing an overview of required sample material (biobanking) and measurement techniques for the different biomarkers. All the aging biomarkers discussed above can readily be measured in the patient's blood by the use of robust, well-established methods. A comprehensive description of all the technical procedures would go beyond the scope of this review, but more specific details can be found in the references cited herein. However, it should be emphasized that there are specific requirements for sample collection and processing, depending on the type of biomarker assay(s) that is (are) to be performed and that certain constraints should be taken into consideration *before* initiation of the biobanking project. It is of critical importance to decide which biomarkers are of interest in the context of a specific study, and to compose a relevant biomarker assay panel in the initial stage of the project (i.e. at the time of study protocol development) in order to ensure that sample material is properly collected in sufficient amounts. Table 3 gives an overview of sample collection, processing and analysis methods for the different aging biomarkers that were described above.

8. Conclusions/Future Challenges

Due to recent advances in biology and genomics, it is becoming possible to personalize an individual's cancer treatment based on the molecular characteristics of the tumor. In the elderly, however, host factors also become increasingly important for treatment decisions, especially relating to the risks of toxicity in compromised individuals, balanced with potential benefits. Personalized medicine in oncology should thus not only focus on treatment at the tumor level, but also personalization towards the individual with his/her specific general health status. Given the variability in health conditions among elderly people, undertreatment due to fear of toxicity is a frequent problem in older patients with cancer. On the other hand, expensive therapies with serious side-effects are sometimes given to patients who do not tolerate and/or do not benefit from them. Moreover, chemotherapy by itself is suspected to accelerate the process of aging and, more specifically, immunosenescence. Therefore, it is of the utmost importance to consider the aspect of aging in the context of cancer treatment. It should be emphasized though, that "aging" is used here in the sense of patient's health status, life expectancy, vulnerability, ability to tolerate treatment and not chronological age per se.

Retrospective and some prospective evidence supports the notion that several biomarkers might be able to predict morbidity and mortality in older people in general, and by extrapolation in older patients with cancer too. This information is crucial for therapeutic strategy planning and could influence treatment decision towards a more tailored approach that will hopefully improve quality of life, compliance and outcome. However, many questions remain to be answered. The ability of these potential "aging biomarkers" in envisaging patients' prognosis and selecting patients with cancer suitable for chemotherapy is mostly based on retrospective data and should be confirmed in prospective clinical trials. Moreover, the studies cited here show that these 'aging' markers are expressed at different levels in older vs. younger subjects , but the real question is whether these markers discriminate between older people who are fit and those who are less fit. Limited longitudinal studies in the very elderly suggest that this is indeed the case, but clearly well-designed and conducted validation studies are essential. Most importantly of course, the majority of data is derived from the general older population and must be confirmed in older patients with cancer. The impact of chemotherapy and/or radiation on the senescence process is likely to alter the predictive capacity of several biomarkers. However, the majority of anticancer therapies are associated with significant toxicity; therefore the ability to identify patient's biological age is critical. Another issue is the large quantity of markers that have been studied and proposed. Is one biomarker clearly better than the others or should different

Table 3 – Overview of sample collection and analysis methods for the different aging biomarkers.

Biomarker	Sample material	Blood collection, processing and storage	Measuring technique(s)	Ref.
Circulating cytokines/ chemokines and CRP	Plasma or serum (serum possible for some but not all)	EDTA (for plasma) or SST II (for serum) blood sample: centrifuge within 20–60 min, isolate plasma/serum, store frozen at −80 °C	-ELISA-multiplex assays (BioPlex, Luminex)	43,150,151
Mean leukocyte telomere length	Leukocyte DNA	EDTA blood sample: centrifuge within 1 h and freeze precipitated cell fraction for future DNA extraction	-qPCR (T/S method)-Southern blotting (non-radioactiveTRF method)	152,153
CRAMP, EF-1á, stathmin	Plasma or serum (serum slightly better)	EDTA (for plasma) or SST II (for serum) blood sample: centrifuge within 20–60 min, isolate plasma/serum, store frozen at −80 °C	-ELISA	86,132
Chitinase (chitotriosidase) activity	Plasma	EDTA blood sample: centrifuge within 1 h, isolate plasma, store frozen at −80 °C	-Fluorimetric enzyme assay	86,132
Circulating IGF-1 (ISS signaling pathway)	Plasma or serum	EDTA (for plasma) or SST II (for serum) blood sample: centrifuge within 20–60 min, isolate plasma/serum, store frozen at −80 °C	-ELISA	154
Genetic variation in aging-related genes	Germline DNA	EDTA blood sample: centrifuge within 1 h and freeze cell pellet for later DNA extraction	-Genotyping of single nucleotide polymorphisms (SNP) by iPLEX technology (Sequenom)	155
Changes in immune cell subsets	PBMC	Fresh EDTA blood sample: Isolate PBMC by density gradient centrifugation; freeze cells at −80 °C for future phenotyping experiments	-Antibody stainings-Multiparameter flow cytometry	121
$p16^{INK4a}$ expression in T cells	RNA from T lymphocytes	Fresh EDTA blood sample: Isolate T cells by density gradient centrifugation in combination with negative antibody selection; freeze cells at −80 °C for future RNA extraction	-RT-qPCR	156
Gene expression in PBMC	Total leukocyte RNA	PAXgene tube: incubate 2 h store frozen (−20 °C) for future RNA extraction	-Microarray analysis (initial screening)-RT-qPCR (validation)	157
Oxidative stress: 8-iso-Prostaglandin F2	Plasma	EDTA blood sample: centrifuge within 1 h, isolate plasma, store frozen at −80 °C	-Gas chromatography–mass spectrometry (GC–MS)-Competitive enzyme immunoassay (EIA)	158
Oxidative stress: 8-hydroxy-2′-deoxy-guanosine	Plasma	EDTA blood sample: centrifuge within 1 h, isolate plasma, store frozen at −80 °C	-Gas chromatography–mass spectrometry (GC–MS)-High-performance liquid chromatography (HPLC)-Competitive enzyme immunoassay (EIA)	159,160
miRNA expression profiling	Plasma or serum	EDTA (for plasma) or SST II (for serum) blood sample: centrifuge within 20–60 min, isolate plasma/serum, store frozen at −80 °C	-RT-qPCR (Exiqon assays)	161,162

ones be used in different subgroups of older patients? Are these markers any better at predicting relevant outcomes (death from another cause/treatment toxicity) than clinical measures (e.g. CGA; assessments of co-morbidities or functionality [ADL, IADL]) which are cheaper (and maybe easier although CGA is also time consuming) to measure. It is most likely that clusters of parameters will need to be measured, rather than single markers. Thus, given the complexity of the senescence process, using a (selected) combination of markers might be the best approach, and possibly clinical factors from CGA could be integrated as well, in order to generate some kind of 'aging/senescence index' which could aid in clinical decision making. Another important question is the feasibility of having all these markers tested in clinical practice. Recently, the European Organisation for Research and Treatment of Cancer (EORTC) Elderly Task Force (ETF) has initiated an aging biomarker program. Biological material will be collected in patients participating in EORTC-ETF clinical trials. Candidate biomarkers cover different aspects of aging biology and include leukocyte telomere length, p16INK4a expression in T lymphocytes, immunosenescence markers, oxidative stress markers, circulating inflammatory mediators, genetic variability in aging/longevity-related genes, and miRNA expression. The purpose of this program is to evaluate the feasibility of this approach and validate the ability of this panel of candidate aging biomarkers to determine a person's "biological age", provide important information on life expectancy, determine the reserve capacities of patients as well as their chance of tolerating therapy or the risk of suffering severe toxicity. It is of major importance to study (potential) aging biomarkers in correlation with age at diagnosis, geriatric assessment, disease characteristics and (cancer-specific) survival. Even more important is their value in predicting development of frailty, severe toxicity and decline of functionality when receiving particular therapies. This would allow adaptation of therapies based on the aging biomarker profile before starting therapy and is expected to lead to better outcomes and better QoL for individual patients.

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The authors declare no competing financial interests.

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