Cancer Treatment and Research
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Biological Basis of Geriatric Oncology

edited by
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BIOLOGICAL BASIS OF GERIATRIC ONCOLOGY

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FOREWORD

The population of Western countries is aging, and cancer in older aged persons is becoming increasingly common. The management of these neoplasms is a novel problem. Direct information on the outcome of cancer prevention and of cytotoxic chemotherapy in older individuals is scarce, especially for those aged 80 and over, and it is not clear whether the same process should direct medical decisions in younger and older persons. It is reasonable to assume that the benefits of cancer prevention and treatment diminish and the dangers increase with age. The expected gains from cancer treatment may be lessened by shorter life expectancy. The risk of therapeutic complications may be increased and the consequences of these complications may become more serious due to limited functional reserve of multiple organ-systems, and fading social support and economic resources. In addition, the biology of cancer may change with the age of the patient, due to a series of events that have been clarified only in part. For example, the prevalence of Multidrug Resistance in Acute Myelogenous Leukemia is much higher for patients over 60, which make the treatment less effective and the risk of treatment-related deaths higher. At the same time, the risk of local recurrence of breast cancer after partial mastectomy declines with age, indicating a more indolent disease.

Several publications, including books, review articles and original studies, related to cancer in the elderly have appeared during the last ten years and have highlighted important points that have become widely accepted:

- Age by itself is not and should never be a contraindication to cancer management, including prevention and treatment.
- The management of cancer in the older person should be individualized according to individual life expectancy, treatment tolerance, and risk of experiencing the complications of cancer including death, disability, and discomfort.

- A number of simple provisions may ameliorate the complications of cytotoxic chemotherapy and allow the administration of full doses of treatment. These provisions include prophylaxis of neutropenic infections, avoidance of severe anemia, timely management of mucositis, and provision of adequate home care giving.

The practical application of these directions remains somehow controversial however, as the methods to estimate life expectancy, functional reserve, and tumor behavior are poorly defined. The main goal of this book is to provide a simple blueprint enabling the practitioners of oncology, geriatrics, and primary care to decide when a patient may or may not benefit from cancer prevention and treatment. Based on the current knowledge of the biology of aging and cancer, the books examines several facets of patient assessment, including function, comorbidity, physical performance and laboratory tests, as well as the way these different forms of assessment may be integrated in medical decisions. At the meantime, the book explores future possibilities for understanding the interaction of aging and cancer biology and for predicting these interactions, and provides a rationale for clinical trials of chemoprevention of cancer in the older person by unraveling the mechanisms that associate aging and carcinogenesis. Some of these mechanisms, including the genomic changes of age, are predictable, while others, including proliferative senescence, are counter-intuitive, and open new, unsuspected opportunities for intervention. Aware of the rapid evolution of the field, we wanted for this book to become an expandable and adaptable frame of reference, able to accommodate new information and still able to direct the practitioner in the management of older individuals even when the current information will be outdated. The emphasis on current research directions in the biology of aging, of cancer, and of the hemopoietic system that is intimately connected to the management of cancer, should make the reader attuned to new developments and allow the reader to rapidly incorporate these developments into clinical thinking.

Another important goal of this book is to highlight the important lessons coming from the study of aging that may be collapsed into two points:

- To a large extent, the study of aging involves a movement from the bedside to the bench, which is directly opposed to the current trend of oncology. As underlined in the initial chapter, epidemiology is the main clue to the biological interactions of cancer and aging: epidemiology and clinical observation are still the main source for experimental hypothesis.
- Due to the scarcity of information, the study of geriatric oncology requires acceptance of some degree of uncertainty. In clinical practice this involves attention to unexpected and unpredictable occurrences; in clinical trials this involves readiness to accommodate a number of unknown parameters.

The best opportunity for real progresses in the field may come from the integration of these points in clinical practice and clinical research.

The third and final goal of this book is to provide an updated and practical research handbook for the increasingly large host of young investigators who want to become involved in the field. The need for such handbook is revealed by a number of recent initiatives aimed to promote research in geriatric oncology. Among them we would like to highlight the issuance of a RFA for program grants in geriatric oncology by a combined NCI/NIA effort, and the institution of a number of fellowships in geriatric oncology through a grant of the Hartford Foundation to the American Society of Clinical Oncology.

In addition to all excellent collaborators of the book, we would like to thank the numerous friends and colleagues who have been engaged with us in this adventure of geriatric oncology during the last ten years, and in particular, we would like to acknowledge the leadership of Rosemary Yancik, Ph.D., who single-handedly generated the field more than two decades ago, and the members of the Senior Adult Oncology Program at the H. Lee Moffitt Cancer Center, to whom this book is dedicated.

Lodovico Balducci M.D.

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Chapter 1

EPIDEMIOLOGY OF CANCER AND AGING

Lodovico Balducci and Matti Aapro

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Epidemiology provides the initial clue to causes and mechanisms of diseases. It is well known that age is a risk factor for most common cancer and that incidence and prevalence of cancer increase with age[1]. In this chapter we explore the epidemiology of cancer and aging, in an attempt to understand the biologic interactions of these processes. In particular, we address the following questions:

1. Does aging enhance the susceptibility of older individuals to environmental carcinogens?

2. Is aging associated with increased risk of multiple malignancies?

3. Does the clinical behavior of cancer change with age?

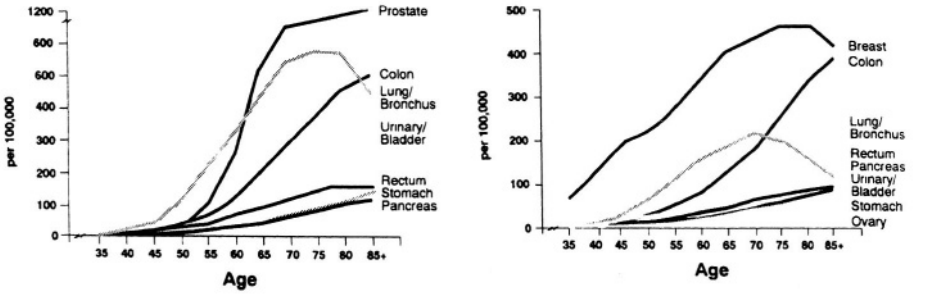
4. Does cancer increase the risk of death of older individuals?

In conclusion we will examine the clinical implications of these questions and propose a research agenda aimed to improve the control of cancer in the older aged person.

1. AGE AND CARCINOGENESIS

The incidence of common cancers increases with age (Figure 1). This association is universal [2] and is observed with the aging of any population around the world. A clear explanation of this phenomenon is the time-length of carcinogenesis, a stepwise process involving the activation of cellular oncogenes, and the suppression of anti-proliferative genes (anti-oncogenes) [3]. It is reasonable to assume that the duration of carcinogenesis reflects the number of stages involved in the pathogenesis of different tumors, and that this number be highest for tumors whose incidence peaks late in life, such as adenocarcinoma of the prostate and of the large bowel, or non-melanomatous skin cancer [3]. In the era of chemoprevention and recognition and elimination of environmental carcinogens, an alternative possibility should be considered. These interventions may cause the prolongation of one or more carcinogenic steps and, in so doing; they may delay the development of cancer. For example, the incidence of lung cancer has decreased for individuals less than 60, while it has increased for older individuals [4]. As a result, the peak incidence of lung cancer has become more and more delayed. Interestingly, these changes have paralleled the incidence of smoking cessation in the Western population. In this case it is reasonable to assume that the length of carcinogenesis has increased as a result of a prolongation of the late carcinogenic stages, from reduced intensity of exposure to tobacco smoke [3]. If this hypothesis is correct, one may expect to see a progressive delay in the appearance of common cancer and an increased incidence of neoplasia in advanced ages.

Figure 1. The incidence of common cancers increases with age.



The duration of carcinogenesis may not account completely for association of cancer and aging. . The incidence of some neoplasms, such as prostate and non-melanomatous skin cancer increases more rapidly with age, than it would be expected from the time-length of carcinogenesis alone [3]. These findings suggest that the concentration of cells in advanced carcinogenic stages increases with the age of an organism, enhancing the susceptibility of older individuals to environmental carcinogens [3]. This possibility is supported by a host of studies of experimental carcinogenesis, summarized in another chapter of this book [3] and also by epidemiologic observations [5-9]. Barbone et al reported the risk of lung cancer after exposure to an environmental pollutant in the Italian city of Trieste increased with the age of the subject at the time of exposure [6]. Since 1970, the incidence of non-Hodgkin's lymphoma has increased 80% for individuals 60 and over, and that of malignant brain tumors seven fold (or 700%) for individuals 70 and older [8, 9]. It is tempting to infer that older individuals develop cancer after exposure to new environmental carcinogens earlier than the younger ones, because of increased susceptibility to these substances. In other words, older subjects may represent a natural monitoring system for new carcinogens. Unfortunately this hypothesis may have proven true, at least in the case of brain tumors, as the incidence of these neoplasms is now increasing also for individuals aged 50 and older [8].

For completeness, other biological changes of aging, beside advanced carcinogenesis, may favor the development of cancer. Immune-senescence may facilitate the growth of highly immunogenic tumors [10], while proliferative senescence may result in loss of cellular apoptosis, and the production of tumor growth factors and proteolytic enzymes that promote the growth and the spreading of cancer respectively [11].

Does the incidence of cancer increase indefinitely with age? The answer to these question as become highly relevant with the progressive aging of the Western population and with the expansion of the oldest segment of the population (those 85 and older), that is increasing more rapidly than any other segment. [12]. The observations of Stanta et al, who performed more than 350 autopsies of individuals aged 95 and older and in more than 100 aged 100 and older suggest that beyond a certain age the incidence of cancer might decrease [13]. These authors reported that not only the incidence of cancer as cause of death and the incidence of clinical cancer, but also the incidence of occult cancer decreased after age 95. Of interest, the decline in cancer was associated with increased incidence of sarcopenia, and atrophy of multiple tissues, which suggest that at the upper extreme of age the anabolic processes are reduced to an extent that they cannot support the rapid growth of neoplastic tissues. An alternative possibility is that genes involved in longevity may also be involved in protection from cancer.

2. AGE AND MULTIPLE NEOPLASMS

As aging is a risk factor for cancer, it is reasonable to ask whether the incidence of multiple primary malignancies is more common in older persons and in particular whether an aging phenotype of increased cancer risk may be defined. The recognition of such phenotype would have important practical consequences, which include the ability to target certain individuals for cancer prevention and new insight in the molecular pathogenesis of cancer. Luciani and Balducci have considered two alternative hypotheses (Figure 2) [14]. According to both hypotheses the incidence of multiple primary malignancies increases with age. In model A this increment reflects only the general risk of cancer associated with aging, whereas in model B previous history of cancer is itself a risk factor for new neoplasms. Model B implies an aging phenotype associated with increased risk of multiple malignancies. After review of the literature, the authors concluded that model A was more likely that model B. Absolute conclusions are not possible, however, due to the limitation of existing data (Table 1).
Universal consensus is wanted for the definition of multiple primary malignancies. In the majority of study series the definition of Warren and Gates has been utilized [15]. This implies the fulfillment of two conditions: each tumor must present an independent clinical and pathologic picture and the possibility that one neoplasm be a metastasis of the other should be excluded. A number of serious limitations related to this definition are self-evident. First, it fails to distinguish between clinically relevant and irrelevant neoplasms as it is based on autopsy studies. Second it fails to address the issues related to multifocal tumors occurring in the same organ, that are defined by two questions: how can it be established that multifocal tumors are distinct tumors; and should multifocal tumors be considered multiple primary malignancies.

The development of multifocal tumors is a consequence of “field carcinogenesis “ implying that the same tissue may give origin to multiple neoplasms, as the whole tissue has been exposed to the same carcinogen for the same duration of time [16]. The development of multiple tumors in breast, large bowel, head and neck and bronchus support this theory [16]. The distinction of different tumors arising from the same organ may be problematic. The recognition of histologic differences (for example squamous cell carcinoma, adenocarcinoma or neuro-endocrine tumors) is by itself not a definitive proof of distinction, as it is well known that the same

Figure 2. Alternative hypotheses on the increased incidence of multiple primary malignancies with age [14]. Model A reflects only the general risk of cancer associated with aging. Model B implies an aging phenotype associated with increased risk of multiple malignancies.

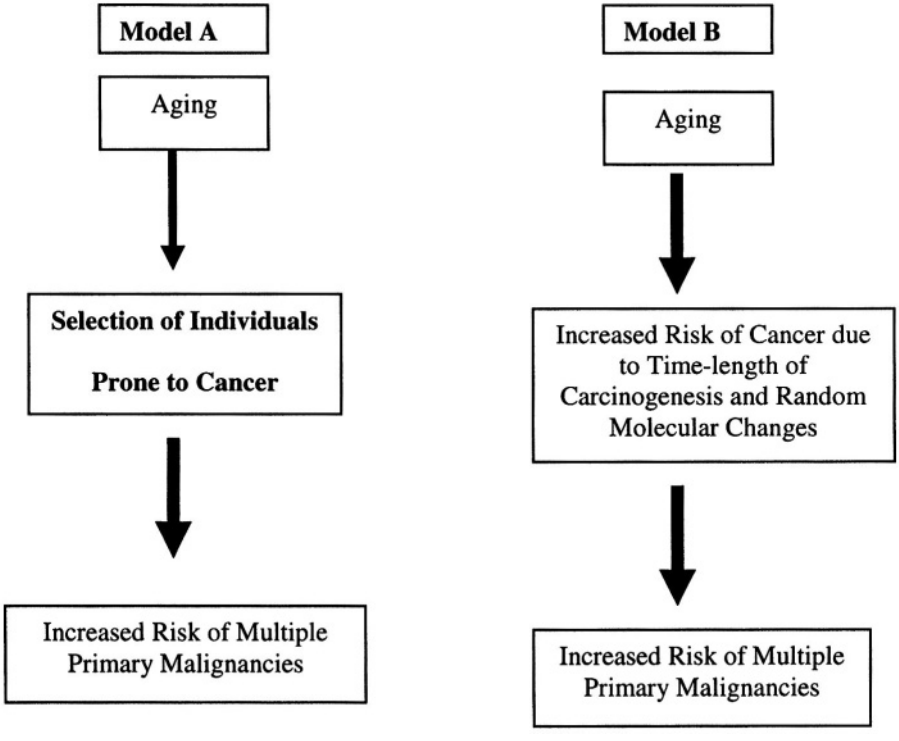


Table 1. Methodological difficulties related to the diagnosis of multiple primary malignancies

- Definition
- Clinical and pathologic recognition
- Influence of previous cancer treatment
- Selection bias
- Limitation of existing sources of data
- Tumor registries
- Autopsy series

epithelial stem cell can give origin to different neoplastic phenotypes [14] Mortel proposed that two tumors arising in the same tissue be considered independent when the tissue separating the two neoplasms does not show

neoplastic infiltration [17]. Though helpful, this criterion appears inadequate on two accounts: it relies on the correctness of individual observations, and it excludes the possibility of surface metastases.

Last but not least, there is an age-specific problem related to the association of age with multiple primary malignancies. This involves the decision whether one should consider that age at which the first or the subsequent tumors did occur. Conceptually, it appears reasonable to consider affected by age-related multiple primary malignancies only those patients whose first cancer was diagnosed during adulthood, but we recognize that this proposal only shifts the problem to the definition of adulthood.

One common problem in the definition of multiple primary malignancies is whether the subsequent neoplasms are metastases of the initial one. This difference can be established with absolute certainty only when the tissue of origin of the original and subsequent tumor is different (for example epithelial and mesenchymal neoplasms). Electron microscopy and immune-histochemistry have also helped to identify tumors of origin from different tissues [14]. In the case of some tumors, specific characteristics, such as the presence of hormone receptors in breast cancer allow establishing whether a tumor occurring in different organs is a metastasis of the original neoplasm.

The treatment of cancer may be itself a cause of new cancer, and enhance the risk of a second malignancy in patients who have received antineoplastic treatment. The association of acute myelogenous leukemia with cytotoxic chemotherapy [18] is well known. Cervical cancer has been associated with an increased risk of cancer of the bladder, small intestine, ovary, bones, and of multiple myeloma, but only in patients who had been treated with radiation therapy [19].

A number of selection biases may convey the impression that multiple primary malignancies after diagnosis of an initial cancer. Undoubtedly, patients with a diagnosis of cancer do receive more diagnostic tests, to stage the initial cancer and to establish the presence of recurrences. These tests may reveal concomitant occult malignancies. For example, staging of non-Hodgkin's lymphoma led to the diagnosis of a number of unsuspected renal cell carcinomas [14]. In addition to these diagnostic biases, there is a survival bias. That is the patients who survive the first cancer are more likely to carry the diagnosis of subsequent cancers as a consequence of the fact that they live longer [14]. Though not properly a "selection bias" another source of error may be the changing incidence of certain malignancies with time. For example, non-Hodgkin's lymphoma appeared more common in patients with previous diagnosis of renal cell carcinoma, before it was realized that this association reflected the increased incidence of lymphoma in the general population during that period of time [20].

The main source of information on multiple primary malignancies is tumor registries and autopsy studies. Tumor registry studies are cohort studies, whose value varies with the quality of the registry as well as with the quality of cancer care provided during the time covered by the registry. For example, studies performed during a time when women received routine mammographic screening are more likely to demonstrate the association of breast cancer with other malignancies, because breast cancer was diagnosed at an earlier stage and associated with a more prolonged survival. In general tumor registry studies showed that the risk of second malignancies increased with the duration of survival since the diagnosis of the initial neoplasm [14]. Autopsy studies are by there own nature selective, as they depend on the ability of physicians to obtain autopsy and on the willingness of patients' family to allow the procedure. These cross-sectional studies showed that the prevalence of multiple malignancies increased with the patient's age, but it was consistent with the general risk of cancer for that age [14]. In conclusion, both autopsy and registry studies demonstrated that the diagnosis of multiple primary malignancy was more likely in patients of advanced age, but age was not a risk factor for increased risk of multiple primary malignancies. These studies favored model B over model A in figure 1. It should be noticed that increased likelihood of association was found between certain types of cancer including smoking related cancer [16], papillary cancer of the kidney and cancer of the bladder and of the prostate [21], and breast and uterine cancer [22]. The latter was observed only in women aged 70 and older.

The increased possibility of multiple primary malignancies in older individuals has important clinical consequences:

- The development of a new lesion in patients with history of cancer should be investigated to rule out the possibility of a new and curable malignancy and should not be dismissed as a recurrence off the previous cancer. .
- Previous history of cancer should not prevent aggressive treatment of new cancer. It is not unusual for an older individual to carry a diagnosis of two or more primary malignancies, all of which have been curable.

3. AGE AND NATURAL HISTORY OF CANCER

It is well established that the biology of some malignancies may change with the age of the patient due to at least two underlying mechanisms (Table 2). One may think metaphorically of the tumor as a plant, whose growth is affected by changes in the seed (the neoplastic cell) and the soil (the aging tumor host). In the case of AML the seed is responsible for

reduced responsiveness to chemotherapy and decreased likelihood of complete remission after chemotherapy-induced marrow aplasia [23]. A possible explanation for the worse prognosis of NHL in the aged [24] include the fact that aging is associated with increased circulating concentrations of IL-6 [25] one of the most powerful lymphatic growth factors [26]. Both seed and soil may conspire in making breast cancer a more indolent disease in older women: the prevalence of slowly proliferating [27], hormone-responsive tumors increase with the age of the patient, while endocrine senescence and, paradoxically, immune senescence may disfavor its growth. The role of immune senescence has been revealed in a couple of studies showing that the

Table 2. Age and behavior of common malignancies.

Tumor	Change in behavior
Acute myelogenous leukemia (AML)	Less responsive to chemotherapy after age 60 due to higher prevalence of Multi Drug Resistance (MDR). Less likely to yield remission after marrow aplasia, because the pluripotent hemopoietic stem cell may be involved by the disease The course of the disease may be more indolent than in older individuals due to higher prevalence of "smoldering acute leukemia" and "hypoplastic acute leukemia"
Non-Hodgkin's lymphoma, low and intermediate grade	Age may be associated with decreased response rate to chemotherapy and decreased remission duration. The worst prognosis may be due to increased circulating concentrations of Inteleukin-6 in older individuals
Breast Cancer	Diagnosed at more advanced stage in older women. Growth rate appears lower and may be due to a combination of factors, including higher prevalence of slow-growing, hormone-responsive tumors, endocrine senescence and immune senescence
Lung cancer, non small cell	More likely to be diagnosed at an early stage after age 70 Slower growth rate after age 70 Mechanism of change unknown
Ovarian cancer	Prognosis is worse with age, irrespective of stage and of treatment; mechanism unknown
Cancer of the large bowel	No clear relation between age and tumor behavior

growth of primary breast cancer was inversely related to the degree of mononuclear cell infiltration [10, 28], suggesting that these cells produce a cytokine promoting neoplastic growth. The statement that breast cancer becomes more indolent with age contrasts with some reports that age over 75 is associated with more advanced disease and reduced survival [29-31]. The contradiction may be only apparent, as the worst prognosis in women aged 75 and older may reflect lesser utilization of mammographic screening and of adjuvant treatment, and increased risk of mortality from comorbid conditions. Several lines of evidence suggest that breast cancer becomes more indolent with age including reduced risk of life-threatening hepatic and lymphangitic lung metastases, and reduced local recurrence rate after partial mastectomy [32-36].

In the case of non-small cell lung cancer a more indolent course is suggested by reports from different centers that lung cancer presented at an earlier stage in older than in younger individuals [37-39]. These reports may be fraught a referral bias, however, as only older patients with resectable tumors might have been referred to the centers for treatment. It is possible that lung cancer after age 70 involved preferentially ex-smokers, in whom reduced exposure to tobacco smoke resulted in more indolent tumors. While several studies have shown that age is associated with decreased treatment response and survival in women with ovarian cancer, the mechanism of this change has not been clarified[40].

The study of the natural history of cancer relies mainly on old reports, of questionable methodology, as in the last twenty years the majority of cancer patients have received some form of antinoplastic treatment. From a clinical standpoint the critical question is whether there are circumstances in which the management of cancer in older individuals may cause worse complications than the neoplasm itself. Clearly, the natural history of cancer is only one aspect of this decision that involves also the life expectancy and the functional reserve of individual patients [41, 42]. In addition is important to notice that major advances in cancer treatment may have minimized the risk of complications. These include more limited surgery, safer general anesthesia, laser surgery, cryosurgery, radiofrequency tumor ablation, radiosurgery, brachytherapy, conformal field radiation therapy, low dose weakly chemotherapy, and antidotes to chemotherapy-related toxicity, such as hemopoietic growth factors, and targeted therapy. In general, the same treatment of cancer that is beneficial to younger patients appears beneficial to the older ones, albeit to a lesser extent. Though the risk of local recurrence after partial mastectomy decreases with age, radiation therapy improves the chance of breast preservation even for older women [43]. Adjuvant hormonal therapy reduces the risk of breast cancer recurrence and death for women younger than 50 and older than 70[44], while adjuvant chemotherapy may be

beneficial to older post-menopausal women [45]. Likewise, age does not seem to reduce the benefits of adjuvant chemotherapy in patients with stage III cancer of the large bowel[46]. The only situations in which the natural history of cancer may suggest to forgo the use of antineoplastic treatment include smoldering AML and early stage prostate cancer in man aged 70 and older. Though smoldering acute leukemia is an obsolete term, this definition may still be helpful to encompass two conditions: hypoplastic acute leukemia, that is AML with a marrow cellularity lower than 10% and AML associated with Myelodysplasia, with a percentage of blasts in the bone marrow between 20 and 30%, that does not undergo any significant change over three months. In both cases the predominant clinical picture is pancytopenia, the incidence of leukostasis is negligible, cytotoxic chemotherapy is associated with low therapeutic response and high risk of early mortality, while supportive treatment with transfusion of blood products and possible erythropoietin may allow months of quality survival [47]. The value of local treatment of early prostate cancer in patients aged 70 and over has been debated [48]. A study in which patients aged 60 to 75 were randomized to observation and radical prostatectomy demonstrated that surgery was associated with decreased risk of prostate cancer-related deaths, but not overall survival benefits [49, 50].

4. PROFILE OF THE OLDER CANCER PATIENT

Aging is associated with reduced functional reserve of multiple organ systems, increased prevalence of comorbidity, memory disorders, depression, malnutrition, polypharmacy and functional dependence [51]. It is legitimate to ask whether these conditions may interfere with the treatment of cancer and may reduce the patient's life expectancy and tolerance of treatment to the point that treatment is futile or even harmful.

In three studies, cancer patients aged 70 and older had undergone a comprehensive geriatric assessment prior to the institution of treatment, with similar conclusions [52-54]. Some form of functional dependence was present in up to 70% of patients, some form of comorbidity in up to 90%, depression, malnutrition and memory disorders in approximately 20% and polypharmacy in 40%. . A review of the Surveillance, Epidemiology and End Results (SEER) data also revealed that some form of comorbidity was present in the majority of cancer patients aged 65 and older [55]. These studies show the benefits of a comprehensive evaluation of older individuals that allows an estimate of life expectancy and tolerance of treatment, recognition of conditions that should be reversed prior to treatment and the utilization of a common language in the definition of older individuals [56]. As a result of

these studies should be highlighted the need to adjust the doses of chemotherapy to the renal function of older individuals, to investigate anemia, that is a risk factor for mortality, functional dependence, and chemotherapy related toxicity, the management of depression, and the provision of a home caregiver in patients at risk to develop functional dependence during cancer treatment.

Another series of study compared the survival and the general function of older cancer patients with that of individuals of same age without cancer. Diab et al review the SEER breast cancer experience and showed that for women aged 75 and older breast cancer was not associated with a change in survival. Unexpectedly, breast cancer was associated with a more prolonged survival in women aged 80 and older. This observation suggests that breast cancer may affect preferentially women in best general condition, who might have lived even longer if they had not developed breast cancer[57]. This hypothesis is supported by two other studies. Repetto et al compared functional dependence and comorbidity of patients 65 and older with and without cancer admitted to two general hospitals in Italy and found that cancer patients had lower prevalence of both conditions [58]. In a retrospective study of the population of Cusumano, Italy, Ferrucci demonstrated that patients who developed cancer had the highest degree of function and the lowest of comorbidity [59]. Similar conclusions were drawn by Stanta et al from autopsy studies of elderly persons with and without cancer [13].

It is reasonable to surmise that cancer is preferentially a disease of healthy elderly individuals and that the treatment of cancer in these individuals may result in prolongation of survival and quality of life improvement.

CONCLUSIONS

A review of the epidemiology of cancer and age allows concludes:

- Age is a risk factor both for cancer and carcinogenesis, at least up to age 95;

- Multiple primary malignancies are more common in older individuals. In many case each of these neoplasms is amenable to cure or life-prolonging treatment. Possible exceptions include localized low grade prostate cancer in men aged 70 plus and smoldering acute leukemia

- The biological behavior of cancer may be altered with age: in some cases the neoplasm may become more resistant to chemotherapy, in other cases more aggressive and in other cases more indolent;

- Cancer is prevalently a disease of healthy elderly individuals whose life expectancy and quality of life may be compromised by cancer.

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Chapter 2

BIOLOGICAL INTERACTIONS OF AGING AND CARCINOGENESIS

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It is well documented that the incidence of malignant tumors increases progressively with age, both in animals and humans [1-3]. The relationship between aging and cancer is not clear. Considerable controversy surrounds the mechanisms that lead to increased incidence of cancer in the aged. Three major hypotheses have been proposed to explain the association of cancer and age.

The first hypothesis holds this association is a consequence of the duration of carcinogenesis. In other words, the high prevalence of cancer in older individuals simply reflects a more prolonged exposure to carcinogens [4]. The second hypothesis proposes that age-related progressive changes in the internal milieu of the organism may provide an increasingly favorable environment for the induction of new neoplasms and for the growth of already existent, but latent malignant cells [5-9]. These mechanisms may also include proliferative senescence, as the senescent cells loses their ability to undergo apoptosis and produce some factors which stimulate epithelial cells with oncogenic mutations [10]. The third hypothesis proposes that the cancer-prone phenotype of older humans might reflect the combined effects of cumulative mutational load, increased epigenetic gene silencing, telomere dysfunction and altered stromal milieu [11]. The elucidation of causes of an age-related increase in cancer incidence may be the key to a strategy for primary cancer prevention.

1. AGING AND MULTISTAGE MODEL OF CANCER

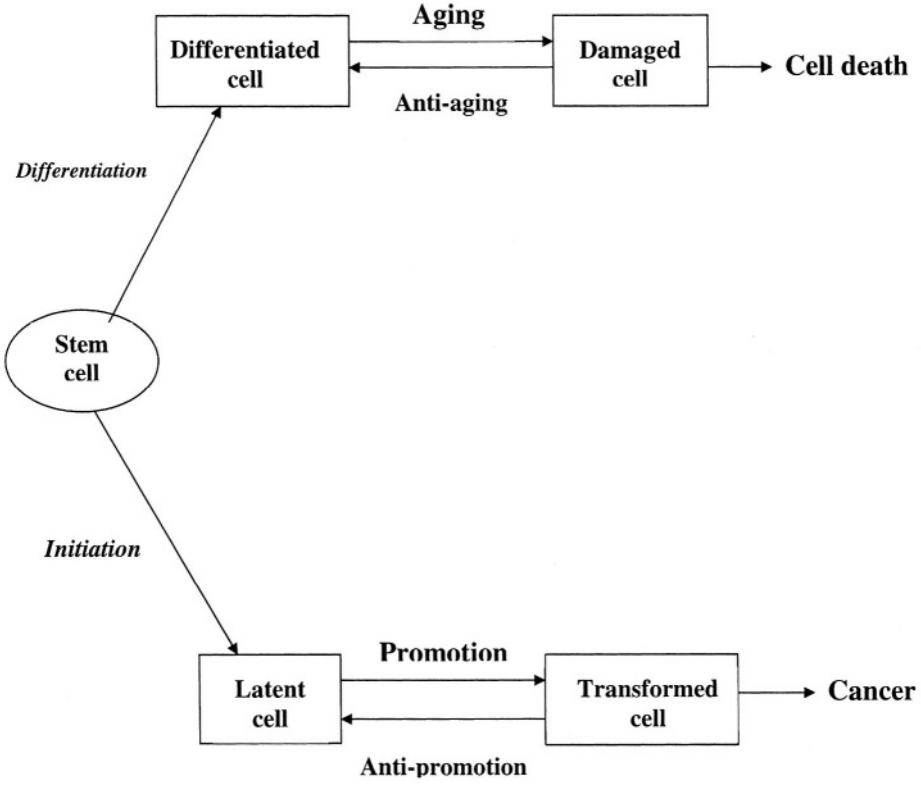
The homeostasis of most tissues is maintained thanks to a pool of stem cells able to reproduce themselves and to differentiate. Cell differentiation is followed by cell death and aging maybe construed as a progressive loss of stem cells to differentiation and death [12]. Another possibility involves the immortalization of the stem cell that is associated with a loss of differentiation and apoptosis. These immortalized stem cells may give origin to a clonal population with a survival advantage over the remaining tissues: this process is carcinogenesis [12,13]. (Figure 1). Both differentiation and death, and immortalization are multi-stage processes. Many steps of carcinogenesis are well-characterized [5,6,14,15] whereas the steps of aging need better recognition and definition [6,16]. Both models of cellular aging and immortalization involve delayed genomic instability that is a transmission of genomic aberrations to distant cellular progenies, accompanied by the occurrence of new aberrations. In one case this process results in cellular death; in the other, in cellular immortalization, and some steps may be shared by the two [16].

Carcinogenesis is a multistage process: neoplastic transformation implies the engagement of a cell through sequential stages, and different agents may affect the transition between contiguous stages [17,18]. Several lines of evidence support this conclusion [19]:

- Histopathology of tumors reveals multiple stages of tumor progression, such as dysplasia and carcinoma *in situ*
- The two-stage model of chemical carcinogenesis in mouse skin shows that different chemicals affect qualitatively different stages in the carcinogenic process
- The existence of individuals with genetic traits manifested by an early occurrence of cancer (e.g., familial retinoblastoma, colon and rectum adenomatosis) suggests that one of the carcinogenic steps is a germ-line mutation, but additional somatic effects are required for neoplastic development
- Mathematical models based on age-specific tumor incidence curves are consistent with the hypothesis that three to seven independent hits (effects of independent carcinogens) are required for tumor development
- Studies with chemical carcinogens in cell cultures reveal that different phenotypic properties of a tumor cell are required for tumor development
- Studies with viral and tumor-derived oncogenes in cell cultures show that neoplastic conversion of normal cells generally requires multiple cooperating oncogenes.

- Transgenic mice that carry activated proto-oncogenes in their germ-line develop focal tumors, which are apparently monoclonal in origin, suggesting that additional somatic events are required for full malignant progression.

Figure 1. Two strategies of stem cell



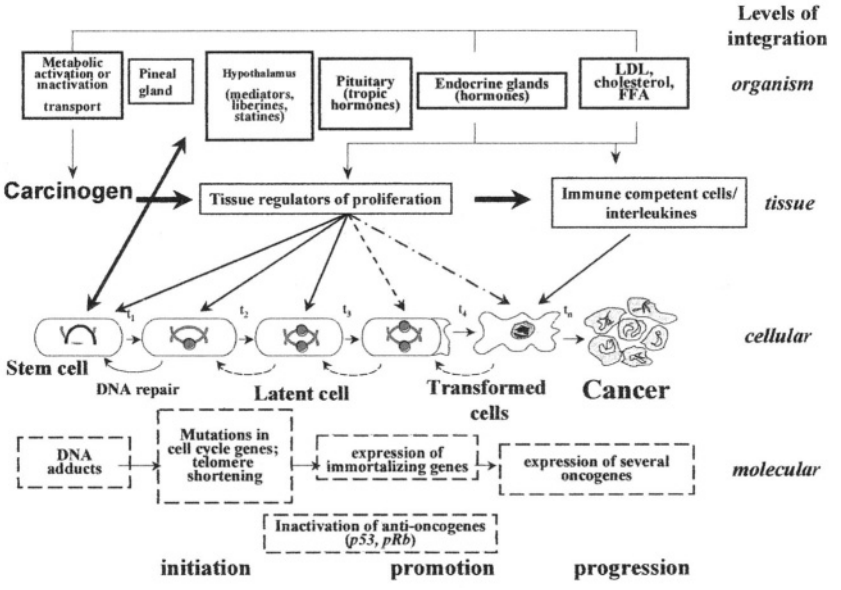
The process of neoplastic development is frequently divided into three operationally defined stages - initiation, promotion and progression. During the first stage of carcinogenesis (initiation) irreversible changes in the genotype of the normal target stem cell leading to its immortality take place. During the initiation the carcinogen or its active metabolite(s) (derived by simple degradation or by active enzymatic process) interacts with nucleic acids leading to mutations in oncogenes and in anti-oncogenes. During the second stage of carcinogenesis (promotion) initiated (latent, immortalized) cell acquires phenotypic features of transformed (malignant) cell, and under the exogenous influence, some of which at least are provided by the neoplastic stroma, tumor progression may occur. A carcinogen affects not only target cells but also influence a lot of factors in the microenvironment of the target cell creating the conditions for promotion of immortalized cell (growth factors, cytokines, immunodepression, biogenic amines, hormonal and metabolic imbalance). Some carcinogens, such as tobacco smoke may effect multiple carcinogenic steps.

Unlike initiation, promotion requires prolonged exposure to the carcinogen and may be reversible to a large extent. The dissection of carcinogenesis into initiation, promotion, and progression is useful as a frame of reference. It should not be assumed, however, that only three carcinogenic stages exist: each stage can be subdivided into multiple substages. Promotion may involve the activation of several enzymes, such as protein kinase C and ornithine decarboxylase; enhanced hexose transport; increased polyamine production, prevention of cell differentiation; and inhibition of cell-to-cell communication [20-21]. It was found that 12-O-tetradecanoylphorbol-13-acetate (TPA), a well-known skin tumor promoter, causes free radical-mediated DNA alterations, such as sister chromatid exchanges and expression of proviruses and retroviruses [22].

Discovery of oncogenes and of their function has provided new insight into the carcinogenic process. One may view carcinogenesis as a "cascade" phenomenon, resulting in serial activation of multiple cellular oncogenes and/or inactivation of tumor-suppressing genes (e.g., p53)[23].

To overcome the obvious limitations of two (three)-stage model, a multistage model of carcinogenesis has been conceived, in which the number of stages is not limited, the stages are envisioned as a continuum, and the influence of factors other than specific carcinogens may be properly accounted for in Figure 2 [24]. The principles of this model are as follows. First, neoplastic transformation involves the transition of target cells through multiple stages, the number of which varies for different neoplasms (with

Figure 2. Integral scheme of carcinogenesis



a minimum of one intermediate stage). Secondly, passage from one stage to another is a stochastic event, the rate of which depends on the dose of a carcinogen that affects the cell. Finally, all cells at any stage of carcinogenesis may enter the next stage independently of each other.

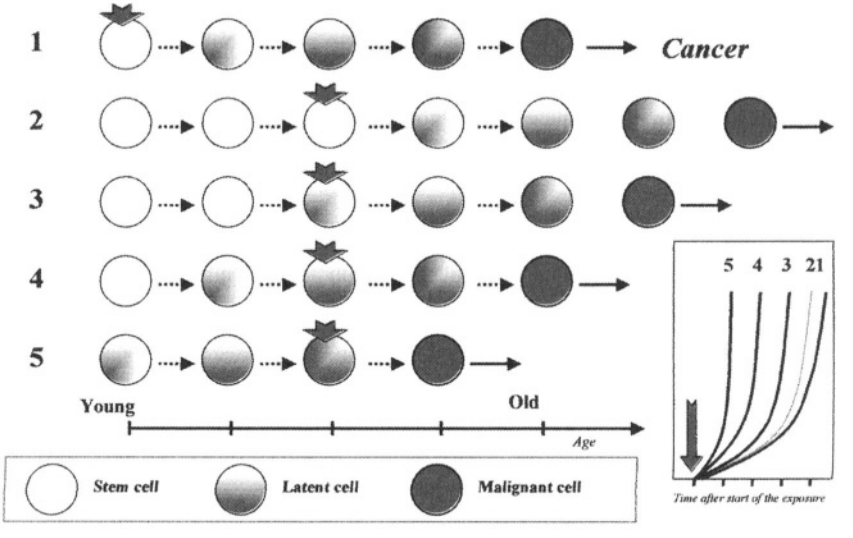
According to this model, the tumor develops only if at least one cell goes through all the necessary stages, and the clonal growth of this cell causes clinical cancer, as a critical volume of neoplastic cells accumulates. In this model, the exact origin of the various stages is ignored and the changes in cell function during the process of carcinogenesis are not assessed. The grade of malignancy is considered to increase with every stage. Various carcinogenic agents (exogenous as well as endogenous) may modulate the process. In addition, some agents act at early stages of carcinogenesis and others at later stages [24]. Epidemiological data, analyzed within the framework of a multi-stage model, have helped to estimate the contribution of various factors to the development of cancer. These factors include the time from the start of carcinogenic exposure, and the age of onset of exposure.

It is worthy to note that in every tissue the number of events occurring in the stem cell before its complete transformation is variable and depends on many factors, in particular the rate of aging of the target tissue and its regulatory system(s) [6,14]. This model is consistent with the analysis of age-related distribution of tumor incidence in different sites in humans and laboratory animals [1,3].

Important differences between early and late-stage carcinogens should be highlighted, to illustrate potential interactions of aging and carcinogenesis. Exposure to early stage carcinogens requires a latent period for the development of cancer. During the latent period the transformed cell goes through the subsequent carcinogenic stages. Clearly, elimination of early-stage carcinogens from the environment will not result in immediate cessation in the incidence of cancer. Carcinogens acting at late stages of carcinogenesis cause the tumor incidence rate to rise after a relatively short period of time. The increased rate of tumor incidence will be reversed almost immediately on cessation of exposure [24].

This risk of cancer after exposure to a carcinogen may be calculated as: $I = (age)^{k-1} - (t)^{k-1}$ where I is the risk of cancer, t is the time from initial exposure to the carcinogen, and k is the number of stages that the target cells have undergone before the exposure to the carcinogen. This formula is based on the assumption that with aging there is a progressive accumulation of partially transformed cells primed to the effect of late-stage carcinogens (Figure 3). Age is considered as a variable because older cells may already present in advanced carcinogenic stages, are primed to the effects of environmental carcinogens and consequently may develop cancer more rapidly and at higher rate when exposed to these substances. A number

Figure 3. The multistage carcinogenesis inducted by single exposure to a carcinogenicagent at different ages.



of factors, including genetic predisposition, oxidative stress, and previous exposure to carcinogens may be responsible of the molecular changes that prime aging cells to environmental carcinogens.

2. EFECT OF AGING ON THE SUSCEPTIBILITY TO CARCINOGENESIS IN VIVO

Animal experiments seem to confirm that there are age related differences in sensitivity to carcinogen in some tissues. Thus, with age, susceptibility to carcinogens in murine mammary gland, small intestine and colon, thyroid, ovarian follicular epithelium decreases, in subcutaneous tissue, cervix uteri and vagina increases and in others (lung, hemopoietic tissues) it remains stable (Table 1). For details see references 1,5-6). Age-related differences in cancer susceptibility have been observed after exposure to the same carcinogens in experimental systems. For example, in female rats exposed to N-nitrosomethylurea (NMU) in doses 10, 20 or 50 mg/kg at the age of 3 month developed mammary carcinomas, tumors of the kidney, ovaries and colon. In contrast to young animals, the rats exposed to the same doses of the carcinogen at the age of 15 months showed a higher frequency of tumors of the corpus and cervix uteri, and a lower frequency of mammary and intestinal adenocarcinomas and tumors of the ovary and kidney [25]. Comparison of the results with the data on DNA alkylation, synthesis and O^6-methylguanine repair obtained on the same model suggests a critical role of age-related proliferative activity changes occurring in the target tissues in the mechanism of age in modifying the effect on carcinogenesis. Obviously, there are no common patterns of age related changes in DNA synthesis and repair or in proliferative activity of different tissues with age [1,5,6].

There are several possible reasons for this wide variation in experimental results. These include factors related to the experimental model and factors related to the tumor-host. Model-related factors involve the characteristics of different carcinogens (direct or indirect action, chemical structure, mechanism of action), route of administration, exposure duration, presence of local and systemic activity, and time of observation. Host-related factors involve animal species, strain, sex, and age. The effective dose of an indirect carcinogen, requiring metabolic activation, may vary significantly in old and young animals, because the activity of the enzymes necessary for carcinogen activation in the liver and/or target tissue(s) may change with age [5,26,27]. Critical factors that determine the susceptibility of a tissue to carcinogenesis include DNA synthesis and proliferative activity of that tissue at the time of carcinogen exposure, and the efficacy of repair of damaged DNA. The

Table 1. Effect of aging on the susceptibility of laboratory rodents to carcinogenesis (Anisimov, 1987; 1998)

Target tissue	Carcinogenic agent	Effect of aging
Skin	DMBA, MCA, BP, TC, UV, β-irradiation	↑
	Fast neutrons, electrone-irradiation	↓
Subcutaneous soft tissues	BP, DMBA, MCA, NMU, polyurethane sponge, Moloney virus	↑
Bone	^{224}Ra, ^{227}Th, ^{239}Pu, radionuclides	=
Vascular vessel	DENA, DMH	=
	Vinylchloride	↑
Hematopoietic tissue	X-rays, γ-irradiation, estrogens	↓
	NMU, pristan	↑=
Mammary gland	DMBA, MCA, NMU, AAF, X-rays	↓
	Estrogens	↑
Uterus	DMH, NMU	↑
Vagina	DMBA	=
Ovary	X-rays, Biskind's operation	↓
Testis	Fast neutrons	↑
Thyroid gland	Fast neutrons, X-rays	↓
Lungs	DENA, DBA, urethane	↓
	NMU, fast neutrons, X-rays	↑
Pleura	Asbestos	↑
Liver	AAF, AFB_1, DMNA, DENA,	↓
	Phenobarbital, CCl_4	↑
Pancreas	NMU	↑
Esophagus	DENA	↓
Forestonach	DENA	↓
Stomach	MNNG	↓
Small intestine	MAMNA	↓
Colon	DMH, MAMNA	↓ (rat)
	DMH, NMU	↑ (mouse)
Kidney	AAF, NMU, DMNA	↓
Bladder	DMBA, BHBNA	↑

Abbreviations: AAF- 2-acetylaminofluorene; AFB_1 – aflatoxin B_1; BHBNA – N-butyl-N-(4-hydroxybutyl)nitrosamine; BP–benzo(a)pyrene; DBA – 1,2,5,6-dibenzanthracene; DENA–N-diethylnitrosamine; DMNA – N-dimethylnitrosamine; DMH- 1,2-dimethylhydrazine; MAMNA – N-methyl-(acethoxymethyl)nitrosamine; MCA – 20-methylcholanthrene; MNNG – N-methyl-N'-nitro-N-nitrosoguanidine; NMU – N-nitrosomethylurea; TC – tobacco smoke condensate; UV- ultra violet irradiation; CCl_4 – carbon tetrachloride; X-rays - Roentgen irradiation.

↑ - increase in incidence of tumors or decrease in tumor latency; ↓ - decrease in incidence of tumors or increase in tumor latency; = no effect.

available data concerning age related changes of these parameters have been discussed elsewhere [1-3,23,28]. Obviously, there are no common patterns of age related changes in DNA synthesis and repair or in proliferative activity of different tissues with age.

The homeostatic regulation of cell numbers in normal tissues reflects a precise balance between cell proliferation and cell death. Programmed cell death (apoptosis) provides a protective mechanism from cancer, by removing senescent, DNA damaged, or diseased cells that could potentially interfere with normal function or lead to neoplastic transformation [23, 29]. Apoptosis plays a substantial role in many other aspects of aging and cancer, including control of the life span of most members of transformed cells, and the rate of growth of tumors [30]. p53 mediated apoptosis was suggested as a safeguard mechanism to prevent cell proliferation induced by oncogene activation [31].

3. AGING AND SUSCEPTIBILITY TO CARCINOGENESIS IN VITRO

Some *in vitro* observations support the suggestion on accumulation in tissues of premalignant cells. Thus, transformed by 24-hours exposure to DMBA, foci in murine bladder epithelium have appeared earlier (on the 40th to the 60th day) and more often (25%) in explants of old (28-30 months) donors in comparison with 100 days and 0.9% in cultures received from five to seven month old mice. A spontaneous transformation of bladder epithelium occurred only in the explants received from old donors [32]. The aging of the tissue donor was associated with increased susceptibility of primary cultures of rat fibroblasts to transformation induced by SV-40 [33]. However rat embryonal fibroblasts were much susceptible to v-*scr* transformation than when they were isolated from an adult rat [34]. Nettesheim et al. [35] reported that the sensitivity of trachea epithelium explants of old animals to chemical carcinogens was lower in comparison to explants from young animals.

Susceptibility to transformation varies during the different stages of proliferative senescence depending on the carcinogen. Thus, young cells are more susceptible to transformation by chemical carcinogens and by low-dose ionizing radiation, susceptibility to ultraviolet radiation is identical throughout the life span of human fibroblasts, whereas susceptibility to a tumor promoter is identical through the cell life span with exception of the final stage, and susceptibility to SV40 is highest during the final stage [36,37].

Thus, experiments both *in vivo* and *in vitro* provide evidence that the age related factors limiting the susceptibility to carcinogens are tissue specific [1,6]. This conclusion may explain, at least in part, both age related

changes in susceptibility to carcinogenesis in target tissues, and organ and tissue variability in age distribution of spontaneous tumor incidence. This conclusion generates a critical question: does the aging accompanied by the accumulation of premalignant lesions in target tissues?

4. EFFECT OF AGING ON THE SUSCEPTIBILITY TO TUMOR PROMOTERS IN VIVO

There is evidence of age-related accumulation of cells that are in the late stage of multi-stage process of carcinogenesis. Numerous experiments support this model. Thus, single skin application with 7,13-dimethylbenz[a]anthracene (DMBA) in mice aged 8 and 48 weeks at doses ranging from 10 to 300 μg caused increased skin papilloma incidence in older mice [38]. Also, the average diameter of the tumors was larger in the older animals. Of particular interest are the experiments using skin transplants. TPA failed to induce tumors in the skin of 2-month-old mice grafted to animals of different ages, but caused the same tumor incidence in the skin of 1-year-old donors irrespective of the recipient's age [39,40]. These results indicate that the age of the target tissue, more than the age of the host, determines susceptibility to late-stage agents. Delaying wounding 16 weeks after initiation with a carcinogen led to a more pronounced skin tumor response compared with delay of only 6 weeks in young mice [41]. Delaying promotion has also been reported to lead to an increased tumor response with the promoters chrysarobin [42] or mezerein [43]. These findings are in agreement with data on age-related decrease in cellular DNA repair capacity in skin [44,45] and increasing *p53* mutation frequency with advancing age in human normal skin [46] and in basal-cell skin carcinomas [47,48]. Post-ultraviolet DNA repair capacity was found to undergo an age-related decline to which corresponded age-related increase in post-ultraviolet mutability in cultured primary skin fibroblasts from normal donors from the first to the tenth decade of life [44]. It was suggested that there was the age-related increase in the number of telomerase positive basal cells in the skin [49]. However in some studies the papilloma response either decreased with age or was the same as the response in younger mice [50-52].

In Tg.AC transgenic (v-*Ha-ras*) mice, skin tumor incidence and multiplicity were strongly age-dependent, increasing with increasing age of the animal when first treated with TPA, or exposed to wounding, or UV-light [53]. The authors suggest that natural developmental changes in keratinocytes are co-opted by the molecular mechanisms that regulate the induction of transgene expression, thus stimulating tumor formation in older Tg.AC mice.

Age-related accumulation of cells in advanced carcinogenic stages may also be inferred by other types of experiments. The mouse model of

hepatocarcinogenesis is very convenient for this purpose because of the availability of strains of animals with different susceptibility to hepatic carcinogenesis. In the liver of highly susceptible mice, the concentration of hepatocytes in advanced stages of carcinogenesis was increased early in life before the exposure to experimental carcinogens [54]. In the liver of F344 rats the number of spontaneous proliferative foci is proportional to the animal age [55,56]. The incidence of proliferative foci and hepatic tumors induced by phenobarbital, carbon tetrachloride or peroxisome proliferators in rodents is also a function of age[55-57].

Another pertinent model involves induction of lymphomas in mice receiving transplants of splenic, thymic and lymphoid cells from syngeneic donors [40]. The incidence of neoplasms was related to the age of the donor, but not to the age of the recipient. Geschickter [58] observed mammary tumor development in estrogen-treated one and 20 month-old rats with a latency period of 9.5 and 3.0 months, respectively. The data on age-related susceptibility to tumor promoters are given in Table 2.

Table 2. Effect of aging on susceptibility of different tissues to tumor promoters in rodents (Anisimov, 1998)

Target tissue	Species	Treatment, agent	Age groups, months	Effect of aging	References
Skin	Mouse	TPA*	4 and 14	↑	39
Liver	Mouse	Phenobarbital	1,5 and 12	↑	55
	Rat	Phenobarbital	1 and 26	↑	56
		Partial hepatectomy + phenobarbital	5 and 18	↑	154)
		CCl_4	1-6 and 12	↑	155
		Clofibrat, nafenopin	3 and 18	↑	57
		Clofibrat, Wy-14643	2,5 and 23	↑	156
Mammary gland	Rat	Estradiol	1 and 20	↑	58
Ovary	Rat	Biskinds' operation**	3 and 14	↑	157

*- 12-O-tetradecanoylphorbol-13-acetate

** Transplantation of the ovary into the spleen after ovaruectomy.

Single intravenous injection of NMU at doses of 10, 20 or 50 mg/kg was administered to female rats aged 3 or 15 months [25]. The NMU carcinogenic dose dependence in different age groups was considered in the context of a multi-stage model. It was calculated that the number of events necessary for complete malignant transformation in 15-month-old rats under the influence of NMU was lower than in three month-old. In this experiment as well as in another sets of experiments in rats and in mice it was shown that tumors developed earlier in older than in younger animals after exposure to the same doses of NMU [14,59-62]. The combined incidences of severe endometrial hyperplasia and adenocarcinomas tended to increase with the increase in intervals between a start of promoting estrasiol treatment after N-nitrosoethylurea initiation in mice [62].

5. EFFECT OF AGING ON TRANSPLANATABLE TUMOR GROWTH

An important question related to the integrated carcinogenic model (Figure 2) concerns age-related changes in tissue microenvironment as these changes may both favor or oppose carcinogenesis in different circumstances. Should aging alter the environment in which tumor develops, the growth rate of transplantable tumors may vary with the age of the tumor recipient [63]. These experiments bypass the effect of age on carcinogenesis itself and explore the role of age-related changes in the organism on the growth and progression of transformed cells. Evaluation criteria for such experiments should include: (a) tumor transplantability, (b) rate of tumor growth, and (c) survival time of tumor bearing animals. The natural history of spontaneous tumors in humans (the rate of tumor doubling, metastasizing potential) and on the survival of cancer patients newly diagnosed at different ages provide information on the effects of age on tumor growth in humans. Available data both in experimental animals and in humans are contradictory and support different effects of age on tumor development (Table 3) [1,6]. In general, an “age effect” may be recognized both in experimental and in human malignancies.

Tissue origin (histogenesis) and immunogenicity of tumor are the principal factors determining age-related differences in tumor growth. There is increasing evidence that age-related changes in tumor microenvironment might play also a significant role. In our experiments, lung-affine cells of rat rhabdomyosarcoma RA-2 were intravenously inoculated into rats of different ages [64]. It was observed that the number of lung tumor colonies was highest in one month-old and 15 month-old animals and lowest in 3 and 12 month-old animals. A positive correlation was found between the number of tumor lung colonies and somatomedine (IGF-1) activity in the lung.

Table 3. Effect of aging on growth of subcutaneously transplanted tumors in rodents (Anismov, 1987, 2003). ↑ - Increase in transplantability and/or the rate of growth and/or decrease in survival time; ↓ - Opposite effects; = No effect.

Tumor	Species	Age at the time of tumor transplantation, months	Effect of aging
Epidermoid carcinoma H.Ep.#3	Mouse	4-8 and 20-23	↑
Squamous-cell cervical carcinoma SCC	Mouse	3 and 12	=
		3 and 18	↑
Melanoma B16	Mouse	3 and 12	↓
		3 and 22	=
		3 and 24	↓
Mammary carcinoma:	Mouse	3,5 and 16,5	↓
Spontanous		10-11 and 21-22	↓
Ehrlich ascite carcinoma		3 and 16,5-18	↑
EMT6		3-4 and 20-28	↑
MAT-21		2 and 4- 5	↓
A-755		3 and 18	↑
64pT		3 and 24	↓
Walker-256	Крыса	2 and 24	↓
Lewis lung carcinoma	Mouse	3 and 18	=
		2 and 24	↑
		3 and 33	↓
Lung carcinoma-1	Mouse	3-8 and 18-23	↑
Hepatoma-22a	Mouse	3 and 14-16	↑
Novikoff's hepatoma	Крыса	4,5 and 27,5	↓
Teratocarcinoma OTT 6050	Mouse	2 and 16	↓
Methylchlanthrene sarcoma	Mouse	6 and 22	↑
		2-3 and 10-21	↑
	Крыса	1-10 and 12-15 8-20 and 29-32	↓ ↑
Fibrosarcoma 1023	Mouse	2 and 4-5	↑
Fibrosarcoma 1591	Mouse	2-6 and 10	↑
Sarcoma 180	Mouse	3 and 18	↑
Osteogenic sarcoma	Mouse	2-3 and 10-17	=
Uterine sarcoma	Mouse	3 and 12	=
Fibrosarcoma	Крыса	4 and 12	↓
Ascitic fibrosarcoma	Крыса	3-4 and 16-18	↑
Mastocytoma P815	Mouse	3 and 25 3-12 and 20-32	↑ ↑
Reticulocell tumor, type A	Mouse	8 and 11-17	↓
Leukemia L1210	Mouse	3 and 11	=
Hemocytoblastoma La	Mouse	3 and 18	=
Myeloma LCP-1	Mouse	2-3 and 19-20	↓

In another experiment, RA-2 cells from a 3-month-old donor were inoculated into 2-3 or 21-23- month-old recipients and 3 weeks later were separately taken from "young" and "old" hosts and transplanted into 3-month-old recipients. The number of lung colonies was significantly decreased in 3-month-old recipients injected with RA-2 cell passed via "old" host [60]. The results obtained suggest the critical role of host and donor microenvironment in lung colony forming potential of RA-2 cells.

McCullough *et al.* [65] have observed that transformed rat hepatocytic cells lines were only weakly tumorigenic following transplantation into the livers of young adult rats. The tumorigenicity of these cell lines increased progressively with the age of the tumor recipients. These results suggest strongly that the tissue microenvironment represents an important determinant in the age-related tumorigenic potential of transformed cells.

Krtolika and Campisi [66] have shown that senescent stromal fibroblasts can stimulate the hyperproliferation and malignant progression of preneoplastic and neoplastic cells in culture. They also tested the ability of senescent fibroblasts to stimulate epithelial cell growth in vivo by inoculation of preneoplastic epithelial cells with presenescent or senescent human fibroblasts into nude mice [67]. None of the tumors when injected alone. Both preneoplastic mouse mammary epithelial cells and preneoplastic human keratinocytes did not form tumors in the presence of presenescent fibroblasts but formed large lethal tumors in the presence of senescent fibroblasts. In the case of human breast cancer cells, senescent fibroblasts markedly stimulated the rate of tumor growth[67].

6. MECHANISMS OF INTERACTION OF AGING AND CARCINOGENESIS

Cancer is a common denomination given to a number of different diseases. Common features to all cancers include [23,68]

- potential immortality of cancer cells due to avoiding apoptosis
- ability to invade surrounding tissues due to reduced sensitivity to signals from neighboring cells aimed to offset proliferation
- cell de-differentiation with re-appearance of some embryonal proteins (e.g. α-fetoprotein) in cytoplasm
- growth signals autonomy, which allows cancer cells to proliferate in absence of outside signals due to only inner growth signals
- release of growth factors and promotion of angiogenesis in tissue, which favor tumor growth and metastasis
- increase in metabolism and number of mitochondria in cancer cells

Gene mutations, as well as changes in regulation of gene expression, which can produce these typical features, were suggested to be key genetic events leading to cancer development[23,68,69]. Down regulation of apoptosis gene, *p53,* as well as upregulation of *myc* and *ras* genes, which may favor excessive proliferation, could be examples of such events [69,70].

Both carcinogenesis and aging are associated with genomic alterations, which may act synergistically in causing cancer [23,68-71]. In particular, three age-related changes in DNA metabolism may favor cell transformation and cancer growth. These changes are genetic instability, DNA hypomethylation, and formation of DNA adducts.

Genetic instability involves activation of genes that are normally suppressed, such as the cellular proto-oncogenes, and/or inactivation of some tumor suppression genes (*p53, Rb,* etc.) [23,31]. DNA hypomethylation is characteristic of aging, as well as of transformed cells. Hypomethylation, a potential mechanism of oncogene activation, may result in spontaneous deamination of cytosine and consequent base transition, i.e., substitution of the pair thymine:adenine. Accumulation of inappropriate base pairs may cause cell transformation by activation of cellular proto-oncogenes [23]. Age-related abnormalities of DNA metabolism may be, to some extent, tissue- and gene-specific. For example, hypomethylation of the c-*myc* proto-oncogene has been found in the hepatocytes, but not in the neurons of old mice [72,73]. Within the same cell, different DNA segments express different degrees of age-related hypomethylation. The uneven distribution of hypomethylation may underlie selective overexpression of proto-oncogenes by senescent cells. For example, the transcription of c-*myc* is progressively increased in the liver but not in the brain of rats between the ages of 4-22 months, whereas the transcription of c-*sis* and c-*src* does not appear to be age-related in any tissues [72,73]. The different extent of DNA abnormalities among aging tissues may account in part for the different susceptibility of these tissues to carcinogens [74,75.]

The damage caused by endogenous oxygen radicals has been proposed as a major contributor to both aging and cancer [76-78]. Endogenous oxidative damage to lipids and proteins increases with age [77,78]. It was shown that oxygen free radicals may induce active mutations of the human c-*Ha-ras* proto-oncogene [78]. The level of one oxidized nucleoside, 8-hydroxy-2'-deoxyguanosine (oh8dG) in the DNA increased with age in liver, kidney, and intestine but remained unchanged within brain and testes of rats, whereas the urinary excretion of the nucleoside decreased with age of rats [79]. A variety of cellular defense systems are involved in protecting cellular macromolecules against devastating action of oxygen-based radicals. These systems include antioxidant enzymes (Cu,Zn- superoxide dismutase (SOD), manganese-containing SOD, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase), some vitamins

(α-tocopherol, ascorbic acid), uric acid and the pineal indole hormone melatonin[80-83].

There is evidence of an age-related accumulation of spontaneous mutations in somatic and germ cells [71]. Accumulation with age of some spontaneous mutations or mutations evoked by endogenous mutagens can induce genome instability and, hence, increase the sensitivity to carcinogens and/or tumor promoters. It has been shown that clonally expanded mtDNA mutations accumulate with age in normal human tissues as well as in human tumors [84,85]. The finding that deleted mtDNA accumulated in human muscle tissue as well as evidence for partially duplicated mtDNA in aged human tissues [85] suggests the important role of clonal expansion of mutant mtDNA in the age-related increase of systemic oxidative stress in the whole organism [86]. A significant trend toward increasing *p53* mutations frequency with advancing age was found in some normal and malignant tissues [46,47]. Simpson [9] suggested that the aging human body accumulates enough mutations to account for multistep carcinogenesis by selection of preexisting mutations. The evidence showed that both genetics of the selected cellular clone and the epigenetics of the selective environment contribute to tumor development[87].

Thus, the data available show that some changes in structure and function of DNA are evolving with natural aging. The character of these changes could vary in different tissues and might cause uneven tissue aging. Dolle et al. [88] using a *lacZ* plasmid transgenic mouse model, determined spectra of spontaneous point mutations in different organs in young and old mice. While similar at a young age, the mutation spectra among these organs were significantly different in old age. The authors stressed that the replicative history *per se* is not the underlying causal factor of age-related organ-specific differences in mutations spectra. Rather, differences in organ function, possibly with association with replicative history, may explain the divergence in mutation spectra during aging. In turn, this may explain both age-related increase in spontaneous tumor incidence and age-related changes in susceptibility to carcinogens in various organs.

Multistage carcinogenesis is accompanied by disturbances in tissue homeostasis and perturbations in nervous, hormonal, and metabolic factors that may favor tumor growth and lessen natural antitumor defenses. The development of these changes depends on the susceptibility of various systems to a carcinogen and on the dose of the carcinogen. Changes in the microenvironment may condition key carcinogenic events and determine the duration of each carcinogenic stage, and sometimes they may even reverse the process of carcinogenesis. These microenvironmental changes influence the proliferation rate of transformed cells together, the total duration of carcinogenesis and, consequently, the latent period of tumor development (Figure 2).

Crosstalk between mesenchyme and epithelium has been described as a known driver of differentiation and development[89,90]. It was shown that changes in stromal behavior can promote epithelial transformation [66,89].

Thus, the data available show that some changes in structure and function of DNA are evolving with natural aging. The character of these changes could vary in different tissues and might cause uneven tissue aging. In turn, this may lead to both age-related increases in spontaneous tumor incidence and age-related changes in susceptibility to carcinogens in various organs. Table 4 summarizes the data available in literature and obtained in our experiments on some hormonal metabolic shifts in the organism and disturbances at tissue and cellular levels observed in natural aging and in different types of carcinogenesis in vivo. Despite incomplete data, it can be seen that there is a similarity between the shifts in aging and carcinogenesis. Carcinogens could be supposed to initiate a normal cell, interacting with its elements on the molecular level, on the one hand, and to produce diverse changes in the organism facilitating promotion and progression of tumor growth, on the other hand.

7. THE ROLE OF THE INSULIN/IGF-1 SIGNALING PATHWAY IN AGING AND CANCER

The potential link between aging and insulin/IGF-1 signaling has attracted substantial attention during last years, on the basis of evidence including age-related increase in incidence of insulin resistance and type 2 diabetes in accelerated aging syndromes and life span extension by caloric restriction in rodents. Concomitant reduction in plasma insulin and plasma glucose levels, which implies increased sensitivity to insulin, emerged as a hallmark of increased longevity [91,92]. Hyperglycemia is an important aging factor involved in generation of advanced glycosylation endproducts (AGEs) [93,94]. There are evidence that hyperinsulinemia favors accumulation of oxidized protein by reducing its degradation as well as facilitates protein oxidation by increasing steady-state level of oxidative stress [95]. Untreated diabetics with elevated glucose levels suffer many manifestations of accelerated aging, such as impaired wound healing, obesity, cataracts, vascular and microvascular damage [8]. It was shown that centenarians have a preserved glucose tolerance and sensitivity to insulin as well as lower degree of oxidative stress as compared to aged persons [96]. It is worthy to note that hyperinsulinemia is an important factor not only in aging but also in the development of cancer [8,97,98].

The intensive investigations in *C. elegans* since 1990's, which have identified insulin signaling components including *daf-2, age-1* and *daf-16* as the genes whose mutations lead to life span extension shed new light on

Table 4. Similarity of changes developing in an organism during natural aging and carcinogenesis (Anisimov, 1997, 2003, with modifications)

Parameters	Aging	Carcinogenesis
	Molecular level	
Free radical generation	Increases	Increases
DNA adducts formation	Increases	Increases
DNA repair efficacy	Decreases	Decreases
DNA hypomethylation	Increases	Increases
Genomic instability	Increases	Increases
Telomere length	Decreases	Increases *
Error protein synthesis	Increases	Increases
Mutation rate	Increases	Increases
Oncogene expression	Increases	Increases
p53 mutations	Increases	Increases
	Cell/tissue level	
Oxidative stress	Increases	Increases
Chromosome aberrations	Increases	Increases
Growth factor production	Deceases	Increases *
Proliferative activity	Decreases	Clonal proliferation*
Focal hyperplasia	Increases	Increases
Apoptosis	Increases	Decreases *
Angiogenesis	Decreases	Increases *
Bioenergetics	Decreases	Anaerobic glycosis *
Cell-to-cell communication	Decreases	Decreases
Latent (dormant) cells number	Increases	Increases
	Systemic/ organism level	
Melatonin circadian rhythm	Damaged	Damaged
Serum melatonin level	Decreases	Decreases
Hypothalamic biogenic amines level	Decreases	Decreases
Hypothalamic threshold of senisitivity to homeostatic inhibition by steroids	Increases	Increases
Tolerance to glucose	Decreases	Decreases
Serum insulin level	Increases	Increases
Susceptibility to insulin	Decreases	Decreases
LDL and cholesterol level	Increases	Increases
Serum glucocorticoid level	Increases	Increases
Fertility	Decreases	Decreases
T-cell immunity	Decreases	Decreases
Cancer risk	Increases	Increases
	Population level	
Cancer incidence	Exponential pattern	Exponential pattern
Progeria	Acceleration	Increases
Exposure to 5-bromodeoxyguanine	Acceleration	Increases
Exposure to ionizing radiation	Acceleration	Increases
Treatment with geroprotectors	Postponement	Decreases or latency increases
Rate at the oldest age	Decreases in mortality	Decrease in incidence

* Related to clonally proliferating malignant cells

molecular mechanisms underlying aging [91,92,99]. In *D. melanogaster,* the mutation of genes operating in the signal transduction from insulin receptor to transcription factor *daf-16* (*age-1, daf-2, CHICO, InR* и др.) are strongly associated with longevity [99,100]. It was demonstrated that FKHR, FKHRL1 and AFX, which are mammalian homologues of *daf-16* forkhead transcription factor, function downstream of insulin signaling and akt/PKB under cellular conditions [101,102].

Daf-2 and InR are structural homologues of tyrosine kinase receptors in vertebrates that include the insulin receptor and the insulin-like growth factor type 1 receptor (IGF-1R). It was shown that in vertebrates the insulin receptor regulates energy metabolism whereas IGF-1R promotes growth. At least three genes (*$Pit1^{dw}$, $Prop1^{dw}$, Ghr*) whose knockout leads to dwarfism have been identified. The expression of these genes is associated with reduced levels of IGF-1 and insulin and increased longevity [103,104]. In Snell and Ames dwarf mice, sexual maturation is delayed, and only few males are fertile, while females are invariably sterile. These mice as well as *$Ghr^{-/-}$* knockout mice have significantly reduced glucose levels and fasting insulin levels, decreased tolerance to glucose and increased sensitivity to insulin which appears to be combined with reduced ability to release glucose in response to acute challenge [91].

Recently, strong support for the role of insulin/IGF-1 signaling pathway in the control of mammalian aging and for the involvement of this pathway in longevity of IGF-1 deficient mice was provided by Hsieh et al [105,106]. It was shown that in the Snell dwarf mice, GH deficiency would lead to reduced insulin secretion and alterations in insulin signaling via InRβ, IRS-1 or IRS-2 and P13K affects genes involved in the control of longevity. The authors concluded that the *Pit1* mutation may result in physiological homeostasis that favors longevity.

Reduction in both glucose and insulin levels as well as an increase in the sensitivity to insulin are a well-documented response to caloric restriction in rodents and monkey [107,108]. It is worthy to note that *$Ghr^{-/-}$* mice have a major increase in the level of insulin receptors [109], while Ames dwarf mice have a smaller increase in insulin receptor and substantially increased amount of insulin receptor substrates IRS-1 and IRS-2 [110]. The development of tumors in Ames dwarf mice was postponed and the incidence was reduced as compared to the control[108].

The crucial event of the effect of caloric restriction is low levels of insulin and IGF-1 and also the increase of insulin sensitivity in rodents [111] as well as in monkeys [112]. Many characteristics of these long-lived mutants and GH-receptor knockout mice resemble those of normal animals exposed to caloric restriction. These characteristics include reduced plasma levels of IGF-1, insulin and glucose, with the consequent reductions in growth and

body size, delayed puberty, and significantly increased sensitivity to insulin action.

Holzenberger et al [113] inactivated the *Igf1r* gene by homologous recombination in mice. It was shown that $Igf1r^{-/-}$ mice dead early in life, whereas heterozygous $Igf1r^{+/-}$ mice live on average 26% longer than wild-type littermates. These mice did not develop dwarfism; their energy metabolism was normal. Food intake physical activity, fertility and reproduction were also unaffected in $Igf1r^{+/-}$ mice. These mice and embryonal fibroblasts derived from them were more resistant to oxidative stress than controls. The spontaneous tumor incidence in the aging cohort of $Igf1r^{+/-}$ mice was similar to that in wild-type controls. At the molecular level, insulin receptor substrate and the *p52* and *p66* isoforms of *Shc,* both main substrates of IGF-1 receptor, showed decreased tyrosine phosphorylation. $p66^{Shc}$ mediated cellular responses to oxidative stress. Two main pathways - the extracellular-signal related kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway – were downregulated in $Igf1r^{+/-}$ mice.

The extension of longevity was observed in fat-specific insulin receptor knockout (FIRKO) mice [114,115]. These animals have reduced fat mass and were protected against age-related obesity and its subsequent metabolic abnormalities including deterioration in glucose tolerance, although their food intake was normal. Both male and female FIRKO mice had increased mean life span (by 18%) with parallel increases in maximum life span. Extended longevity in FIRKO mice was associated with a higher age threshold beyond which age-dependent increase in mortality risk became appreciable and a decreased age-adjusted mortality rate, especially after 36 months of age. In FIRKO mice, the resistance to obesity, despite normal food intake, suggested that metabolic rate is increased, rather than decreased [115]. The authors believe that decreased fat mass could lead to a decrease in oxidative stress. Another possibility is that the increased longevity in these mice is the direct result of altered insulin signaling.

Shimokawa et al. [116] designed a transgenic strain of rats whose GH gene was suppressed by an anti-sense GH transgene. Male rats homozygous for the transgene *(tg/tg)* had a reduced number of pituitary GH cells, a lower plasma concentration of IGF-1, and a dwarf phenotype. Heterozygous rats *(tg/-)* had an intermediate phenotype in plasma IGF-1, food intake, and body weight between *tg/tg* and control (-/-) rats. The life span of *tg/tg* rats was 5 to 10% shorter than -/- rats. In contrast, the life span of *tg/-* rats was 7 to 10% longer than -/- rats. It was found that tumors caused earlier death in *tg/tg* rats; in contrast, *tg/-* rats had reduced nonneoplastic diseases and a prolonged life span. Immunological analysis revealed a smaller population and lower activity of splenic natural killer cells in homozygous *tg/tg* rats. These results

provided evidence that an optimal level of the GH-IGF-1 axis function needs for longevity in mammals.

Recently it was shown that the incidence of mutations in insulin regulatory region (IRE) of APO C-III T-455 C directly correlates with longevity in humans. This is the first evidence showing that mutation located downstream to *daf-16* in insulin signal transduction system is associated with longevity [117]. It is worth noting that centenarians display lower degree of resistance to insulin and lower degree of oxidative stress as compared with elderly persons before 90 years [96]. The authors suggested that centenarians may have been selected for appropriate insulin regulation as well as for the appropriate regulation of tyrosine hydroxylase (TH) gene, whose product is rate limiting in the synthesis of catecholamines, stress-response mediators. It was shown that catecholamines may increase free radical production through induction of the metabolic rate and auto-oxidation in diabetic animals [118]. Recent study on aging parameters of young (up to 39 years) and old (over 70 years) individuals having similar IGF-1 serum levels provides evidence of important role of this peptide for life potential [119]. Roth et al. [120] analyzed data from the Baltimore Longitudinal Study of Aging and reported that survival was greater in men who maintained lower insulin level.

Several years ago, it was suggested to use biguanide antidiabetics as a potential anti-aging treatment [8]. The antidiabetic drugs, phenformin (1-phenylethylbiguanide), buformin (1-butylbiguanide hydrochloride) and metformin (N,N-dimethylbiguanide) were observed to reduce hyperglycemia, improve glucose utilization, reduce free fatty acid utilization, gluconeogenesis, serum lipids, insulin, somatomedin, reduce body weight and decrease metabolic immunodepression both in humans and rodents [8,121,122].

Buformin supplemented at the concentration of 0.1 mg/ml to nutrient medium during the larvae stage and over the life span of *C. elegans* increased the mean life span of the vorms by 23.4% and the maximum life span by 26.1% as compared to the controls [123]. The treatment with phenformin or buformin slightly decreased the body weight of rats, in comparison with the control slow down the age-related switching-off of the reproductive function in female rats prolonged the mean life span of female C3H/Sn mice and LIO rats [1,6,124-128]. Recently it was found that metformin significantly increases the life span of rats (G.S. Roth, personal communication).

Several other effects of treatment with antidiabetic biguamdes related to reproduction and aging, are known from earlier studies. For example, it decreased hypothalamic threshold of the sensitivity to feedback inhibition by estrogens [125-128], which is one of the most important mechanisms regulating age-related decline and switch-off of the reproductive function [125-130]. Treatment with metformin may improve menstrual regularity,

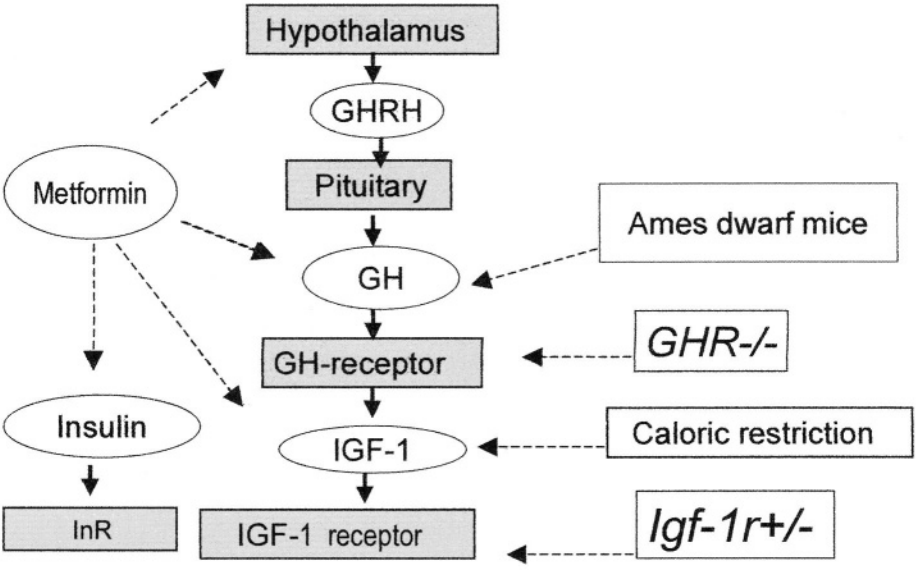
leading to spontaneous ovulation, and enhance the induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome [131]. The treatment with phenformin also decreased hypothalamic threshold sensitivity to feedback regulation by glucocorticoids and by metabolic stimuli (glucose and insulin) [8]. It was recently shown that elements involved in the insulin/IGF-1 signaling pathway are regulated at the expression and/or functional level in the central nervous system. This regulation may play a role in the brain's insulin resistance [132], in the control of ovarian follicular development and ovulation [102], and brain's control of life span [111,133]. Antidiabetic biguanides also alleviated age-related metabolic immunodepression [8]. These mechanisms can be involved in geroprotective effect of biguanides. Treatment with chromium picolinate which elevated the insulin sensitivity in several tissues, including hypothalamus, significantly increased the mean life span and decreased the development of age-related pathology in rats [134]. We hypothesized that antidiabetic biguanides and possibly chromium picolinate regulate thyrosine hydroxylase and insulin/IGF-1 signaling pathway genes both associated with longevity [99,135]. It was shown that the polymorphism at TH-INS locus affects non-insulin dependent type 2 diabetes [136], and is associated with hypothalamic obesity [137], polycystosis ovary syndrome [138], hypertriglyceridemia and atherosclerosis [139].

The anticarcinogenic effect of antidiabetic biguanides has been demonstrated in several models of spontaneous and induced carcinogenesis. The treatment with phenformin normalized the tolerance to glucose and serum insulin and IGF-1 level in rats exposed to intravenous injections of N-nitrosomethylurea (NMU) and inhibited mammary carcinogenesis in these animals [124, 140]. Treatment of rats with 1,2-dimethylhydrazine (DMH) caused the decrease in the level of biogenic amines, particularly of in the hypothalamus, the decrease of glucose tolerance and the increase of the blood level of insulin and triglycerides. Administration of phenformin restored immunological indices and inhibited DMH-induced colon carcinogenesis [140, 141]. The colon 38 adenocarcinoma growth was significantly inhibited in liver-specific IGF-1-deficient mice, whereas injections with recombinant human IGF-1 displayed sufficiently promoted the tumor growth and metastasing [142].

A decrease of glucose utilization was found in the 3-month-old female progeny of rats exposed to NMU on the 21st day of pregnancy [124,140]. Postnatal treatment with biguanides started from the age of 2 months significantly inhibited the development of malignant neurogenic tumors in rats transplacentally exposed to NMU or NEU [143-I44]. In high fat-fed hamsters, the treatment with N-nitrosobis-(2-oxopropyl)amine was followed by the development of pancreatic malignancies in 50% of cases, whereas no

tumors were found in the hamsters treated with the carcinogen and metformin [145].

Figure 4. Proposed effects of metformin, calorie restriction and genetic modifications on insulin/IGF-1 signaling pathway in control of aging. The broken arrows on the figure show the targets for the genetic modifications, calorie restriction and for metformin in IGF-axis.



Thus, anticarcinogenic effect of antidiabetic biguanides has been demonstrated in relation to spontaneous carcinogenesis in mice and rats, in different models of chemical carcinogenesis in mice, rats and hamsters, and in radiation carcinogenesis model in rats. Phenformin administered orally to rodents potentiated the antitumor effect of cytostatic drugs on transplantable tumors [125-127].

The comparative study of 10-years results of metabolic rehabilitation (included fat and carbohydrate dietary restrictions and treatement with biguanides) of cancer patients had suggested increase in the survival of breast and colorectal cancer patients, increase in the length of cancer-free period, decrease in the incidence of metastasis as compared with control patients [122].

Although it is known that free radicals are produced during metabolic reactions, it is largely unknown which factor(s) modulate their production *in vivo*. It has been suggested that hyperinsulinemia may have increase free radicals and therefore promote aging, independent of glycemia[8,94,96]. Plasma

levels of lipid hydroperoxides are higher, and antioxidant vitamins are lower in individuals who are resistant to insulin-stimulated glucose disposal but otherwise glucose tolerant, nonobese, and normotensive [93,95]. There is substantial evidence supporting the hypothesis that selective resistance to insulin-stimulated (muscle) glucose disposal consequent hyperinsulinemia triggers a variety of metabolic effects, likely resulting in accelerated oxidative stress and aging[8,93,95].

The anti-diabetics biguanides inhibit fatty acid oxidation, inhibit gluconeogenesis in the liver, increase the availability of insulin receptors, inhibit monoamine oxidase [121], increase sensitivity of hypothalamo-pituitary complex to negative feedback inhibition, reduce excretion of glucocorticoid metabolites and dehydroepiandrosterone-sulfate [8]. These drugs have been proposed for the prevention of the age-related increase of cancer and atherosclerosis, and for retardation of the aging process [8]. It has been shown that administration of antidiabetic biguanides into patients with hyperlipidemia lowers the level of blood cholesterol, triglycerides, and β-lipoproteins. It also inhibits the development of atherosclerosis, reduces hyperinsulinemia in men with coronary artery disease. It increases hypothalamo-pituitary sensitivity to inhibition by dexamethasone and estrogens, causes restoration of estrous cycle in persistent-estrous old rats, improves cellular immunity in atherosclerotic and cancer patients, lowers blood IGF-1 levels in cancer and atherosclerotic patients with Type IIb hyperlipoproteinemia, [8]. There are data on antioxidative effect of biguanides [133,146] and its neuroprotective activity [147]. It was shown that biguanides inhibits complex I of the respiratory chain in mitochondria that leads to an activation of physiological intracellular inhibition of mitochondrial respiration [148]. Biguanides stimulate a protein kinase cascade inhibiting an expression of transcription factor SREBP-1. An interaction of this factor with cholesterol leads to an increase in transcription of genes coding lipogenesis enzymes and to suppression of free fat acids oxidation. Thus, stimulation of uptake of glucose in tissues by biguanides inhibits lipogenesis and activates oxidation of FFA [149]. It was shown also that in vivo biguanides inhibits an appetite [150,151] and serum levels of leptin and IGF-1 [152]. It was suggested that biguanides regulate energy balance of the organism at the fat tissue level [153]. In general, results of bioguanides effects seem very similar to those of calorie restriction.

Table 5. Comparison of characteristics of rodents subjected to normal aging, caloric restriction, genetic modifications or treatment with antidiabetic biguanides.

Parameters	Aging	Calorie restriction	Dwarf mice	*GH R*$^{-/-}$	*Igf1r*$^{+/-}$	FIRKO	Biguanides
Life span extension	↓	+40-50%	+50%	+46 %	+33%	+18%	+20%
Tolerance to glucose	↓	↑	↓	↓	↑↓[a]	= or ↑	↑
Senstivity to insulin	↓	↑	↑	↑	↑	↑ in fat	↑
Serum level: Insulin	↑	↓	↓	↓	=	↓	↓
GH	↓	↓	Absent	↑	ND	↓	↓
IGF-1	↓	↓	↓	↓	↓	↓	↓
Body size	↑	↓	↓	↓	↓	↓	↓
Body fat content	↑	↓	↑	ND	↑↓	↓	↓
Reproductive function	↓	↓[b]	↓[b]	↓[b]	=[b]	ND	↑
Thyroid function	↓	↓	↓	↓	=	ND	↑
Serum cortico-sterone	↑	↑	=	=	ND	ND	↓
Immune function	↓	↓	= or ↓	ND	ND	ND	↑
Resistance to oxidatve stress	↓	↑	↓	↓	↑	↑	↑
Tumor incidence	↑	↓	= or ↓	=	=	ND	↓

Note: ↓ - decrease; ↑ - increase; = no effect; ND – no available data.

[a] The tolerance to glucose is increased in females but decreased in male.

[b] Reproductive function in relation to normal aging mice.

8. CONCLUSION

The incidence of cancer increases with age in humans and in laboratory animals alike, but patterns of age-related distribution of tumors is different for different tissues and different tumors. Aging may increase or decrease the susceptibility of individual tissues to early carcinogens and usually facilitates promotion and progression of carcinogenesis. Aging may predispose to cancer by two mechanisms: tissue accumulation of cells in late stages of carcinogenesis and alterations in internal homeostasis, in particular, alterations in immune and endocrine system. Increased susceptibility to the effect of late-stage carcinogens is found both in aged animals and elderly humans, as predicted by the multistage model of carcinogenesis. Studies in mammals have led to the suggestion that hyperglycemia and hyperinsulinemia are important factors both in aging and in the development of cancer. Insulin/insulin-like growth factor 1 (IGF-1) signaling molecules that have been linked to longevity include DAF-2 and InR and their homologues in mammals, and inactivation of the corresponding genes followed by the increase in life span in nematodes, fruit flies and mice. It is possible that the life-prolonging effects of caloric restriction are due to decreasing IGF-1 levels. A search of pharmacological modulators of insulin/IGF-1 signaling pathway mimetic effects of life span extending mutations or calorie restriction could be a perspective direction in regulation of longevity. Some old and new observations suggest that antidiabetic biguanides could be promising candidates for both the life span extension and the prevention of cancer.

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Chapter 3

REPLICATIVE SENESCENCE AND CANCER

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1. THE CAUSE OF REPLICATIVE SENESCENCE: TELOMERE SHORTENING

Since the pioneering experiments of Leonard Hayflick in the 1960s it has been known the limited replicative capacity of human cells in culture is very unlikely to be a experimental artifact, but is a reproducible biological phenomenon [1]. However, it was not until it was discovered that the limitation in replicative capacity directly correlates with shortening of telomeres that the notion that it might be a culture artifact was finally laid to rest [2,3]. Telomeres undergo shortening in most dividing human somatic cells because of the lack of telomerase activity that is required for telomere maintenance [4, 5]. The lack of telomerase activity results from the absence of expression of the reverse transcriptase subunit (TERT) of the telomerase ribonucleoprotein complex [6,7]. When cells divide in the absence of telomerase activity about 40-100 bp of the terminal telomeric repeat DNA is not replicated [4, 5]. This amount is a constant for various types of human cells, thus providing a kind of mitotic counter [4,5].

After a normal human cell has divided a certain number of times, further cell division is then blocked by inhibitors of cell proliferation such as $p21^{SDI1/WAF1/CIP1}$ and $p16^{INK4A}$ [8, 9]. When this checkpoint is abrogated by oncoproteins such as SV40 T antigen, which suppress the activity of p53 and pRb, this checkpoint ("Hayflick limit," also termed M1) is bypassed and cells eventually enter a second state, termed crisis or M2 [10]. In this state the much

shorter telomeres undergo end-to-end fusions and initiate chromosomal breakage-fusion cycles that cause the cells to undergo apoptosis. There is massive loss of cells from the culture, whereas in replicative senescence (M1) cells do not die, and are in fact more resistant to apoptosis[11] . About 1 in 10^7 cells undergoes an unknown change that permits escape from crisis/M2 [12]. Such a cell line is said to be "immortal" in the jargon of cell culture, a term that should not be interpreted as implying the reversal of a cellular aging process. In most of these immortalized cell lines the TERT gene has become reactivated [13], but some activate a recombination-based process call ALT (alternative lengthening of telomeres) [14]. Although human fibroblasts can form immortal cell lines in this way by escaping from crisis, they never spontaneously immortalize by escaping from replicative senescence/M1. Most cancer cells that can be grown in culture are also immortal and express TERT. The mechanisms by which cancer cells reactivate or maintain expression of TERT or activate alternate mechanisms for avoiding telomere shortening are largely unknown.

The phenomenon of telomere shortening is clearly the result of the lack of expression of TERT in most normal human somatic cells. Initially it was thought that somatic human cells completely lack telomerase activity and that germ line cells, some stem cells and most cancer cells do have telomerase activity. Subsequently it has become clear that normal human cells do express TERT but that expression is tightly regulated, by processes not yet well understood [15-19]. In some cell types telomerase activity is induced when cells are first isolated from the body and stimulated to divide in culture [20-23]. Curiously, longer-term proliferation is associated with a decline in telomerase activity, sometimes very rapid, so that few long-term cultures of normal human cells have sufficient telomerase activity for telomere maintenance. One type of non-cancer cells that are believed to be immortal are human embryonic stem cells, when they are grown under conditions that prevent differentiation from occurring[24].

Proof that the limitation on indefinite cell division in most human cells results from lack of expression of TERT was obtained by showing that forced expression of hTERT is sufficient to immortalize normal human fibroblasts and retinal pigmented epithelial cells, and is required (although not sufficient by itself) to immortalize keratinocytes and mammary epithelial cells [25, 26]. Immortalization by hTERT is accompanied by increased or stabilized telomere length, but cells retain a normal karyotype [27, 28]. Conversely, some immortal cells suffer telomere shortening and eventual cessation of growth when telomerase is inhibited [29-31].

The second part of the phenomenon of replicative senescence is the state that cells enter as a result of telomere shortening. This state is characterized by altered patterns of gene expression. One well-established biochemical marker for senescent cells, although one of unknown biological significance, is the high level of β-galactosidase enzymatic activity with a pH optimum of 6.0, termed senescence-associated β-galactosidase (SA-β-gal)[32]. In fibroblasts the altered pattern of gene expression resembles that of fibroblasts in inflammation[33]. Of particular significance is the production of proteases that may erode the surrounding extracellular matrix and the production of cytokines that could have effects on neighboring cells[34-37]. Interestingly, other cell types (retinal pigmented epithelial cells and endothelial cells) show different patterns of alteration of gene expression when they reach replicative senescence[33]. The current data are consistent with the hypothesis that the triggering of the block to DNA synthesis that is characteristic of replicative senescence is accompanied by dysregulation of expression of various other genes, and that the pattern of dysregulation will be cell-type specific.

The term "replicative senescence" therefore encompasses two different phenomena: one is the process of telomere shortening, resulting from the lack of expression of TERT, and the second is the cell state that is produced when telomere shortening shuts off further cell division. It is important to distinguish these two processes because they may have quite different implications for the relationship of cancer to aging. One important reason for this statement is that the same set of gene expression changes and molecular markers that occurs in short-telomere senescent cells also occurs in nondividing cells under circumstances that do not involve telomere shortening or cell division. Oxidative stress, radiation, and the ectopic expression of some signal transduction molecules and cyclin-dependent kinase inhibitors can place cells into a senescent state[38-40]. This is often termed "stress-induced senescence." Although both telomere-dependent replicative senescence and stress-induced senescence are termed "terminally nondividing states," the re-initiation of cell division in such cells is possible when genetic interventions are made that can overcome the cell cycle blocks[41].

2. TELOMERE SHORTENING OCCURS IN VIVO IN HUMANS AND OTHER LONG-LIVED SPECIES

It is now well established that many human tissues show shortening of telomeres over the course of the life span. Although that conclusion is now based on direct measurements of telomere length in human tissue

samples, it was earlier observed that proliferative potential in culture of cells from older donors was less than that of cells from younger donors. In the 1970s it was shown that the replicative capacity of human fibroblasts in culture decreases as a function of donor age [42]. It was well known even at the time of these initial observations that there was much variation within each decade of age in the maximal and minimal proliferative capacity of the different cell samples. Subsequently the generality of the observation was challenged by finding that the decrease as a function of donor age applied only to fibroblasts isolated from diabetic and "prediabetic" patients [43], and was not evident in fibroblasts obtained from non-sun exposed skin [44, 45]. However, other sets of data have upheld the original observations [3].

Fibroblasts from older donors also showed a higher level of expression of collagenase, characteristic of replicative senescent cells [46]. Fibroblasts are readily cultured and most cell culture data on the molecular basis for replicative senescence have come from studies on fibroblasts. Unfortunately, fibroblasts as a cell type are not ideal for these kinds of studies. First, the cell population that is isolated is hard to standardize. When fibroblasts are isolated by allowing cells to migrate out from an explant, there is a great deal of selection for what cells form the "starting" culture population (designated as population doubling level zero). Some of the lack of agreement among investigators could result from different isolation techniques, which have provided differing degrees of initial selection. Second, the biology of fibroblasts undoubtedly differs from one organ to another, and even from one part of the skin to another, but these distinctions have not always been considered. Third, most fibroblasts are probably proliferatively quiescent in vivo after maturity and undergo very low rates of cell division. Therefore it would not be surprising if little or no exhaustion of proliferative capacity were observed.

Less ambiguous data are obtained when studies of the effects of donor age on replicative capacity are performed in those cell types where pure populations can be reproducibly isolated from defined sites in the body, and where some cell turnover occurs throughout life. In such cell populations there would be at least the potential for exhaustion of proliferative capacity. Such studies become more powerful when there is an ability to correlate the donor-age cell culture data with direct in vivo data on replicative potential, although this is obviously difficult in humans when such data are from clinical observations rather than direct experimental intervention.

Studies that have been done on non-fibroblast cell populations have shown much larger decreases in proliferative capacity than was observed as a function of donor age in fibroblasts. In some non-fibroblast cell types, many cells in the population isolated from older donors have very limited or no proliferative capacity. Some examples are age-related decrements in

proliferative potential in lens epithelial cells [47, 48], retinal pigmented epithelial cells [49], smooth muscle cells [50-52], osteoblasts [53-55] and adrenocortical cells [56].

Proliferative capacity is closely related to telomere length in endothelial cells. Telomere lengths in endothelial cells decreased as a function of donor age, with a greater decline being observed in cells isolated from the iliac artery in comparison to cells from the thoracic artery [57]. The greater decline in telomere length was observed in the cells had likely undergone more proliferation in vivo, because they resided in a part of the vascular system where blood flow might cause most chronic damage to the endothelium. Unfortunately, because the data are from human specimens, it is difficult to test this hypothesis directly. Skeletal muscle satellite cells can be isolated from human muscle samples and exhibit a limited replicative potential in culture. They show decreasing proliferative potential as a function of donor age and decreased telomere length but muscle fiber nuclei showed stable telomere length [58].

Telomere shortening is observed in bone marrow cells from adult humans in comparison to fetal liver and umbilical cord blood cells [59]. A population enriched in stem cells ($CD34^+$ $CD38^-$) also had shorter telomeres in adults [59]. Lymphocytes show a continuous decline in telomere length with age, consistent with a continuous decline in telomere length in stem cells [60, 61]. The loss of telomere DNA has been measured by a fluorescence technique in lymphocytes and granulocytes from a large number of human donors in the range of 0 to 90 years of age. There was a very striking continuous decline in telomere length, that fits a pattern of a somewhat more accelerated loss of telomere DNA in the age of up to about 1 year followed by a constant linear rate of loss up to the oldest ages studied [62,63]. These data all suggest that telomerase in hematopoietic stem cells is insufficient to maintain telomere length. Direct data for this is difficult to obtain because of the fact that it is now understood that telomerase in many cell types is regulated, as discussed earlier. This fact means that very quiescent stem cells that are not stimulated to divide in culture might be capable of telomerase induction, but this cannot be proved until culture conditions are found that cause them to proliferate and retain stem cell properties [64,65]. Patients with syndromes of increased replication of hematopoietic cells, such as aplastic anemia, including Fanconi's anemia, show variable increases in the rate of telomere shortening, sometimes quite dramatically elevated [66,67].

These examples are consistent with the hypothesis that cell proliferation occurring over the life span of the donor causes telomere shortening; and that cell cultures are then initiated with cells that have a lowered remaining proliferative potential because continued cell division in culture shortens telomeres to a point where replicative senescence occurs. Independent of the question of whether replicative senescent cells affect

tissue function, it is clear that telomere shortening does occur in human tissues in vivo, potentially putting cells ever closer to replicative senescence. It is important to distinguish the phenomenon of telomere shortening from its significance. The significance is still under debate, but the fact that it does indeed occur should not be considered controversial.

The phenomenon of telomere shortening, leading to replicative senescence, has been described in some other mammalian species. For example, bovine cells lose telomeric DNA at each cell division and eventually enter replicative senescence. Expression of hTERT immortalizes bovine cells by preventing telomere erosion [68], as in human cells [25]. The discovery that animals can be cloned from nuclei of bovine cells close to senescence also shows that replicative senescence in this species is not associated with chromosomal abnormalities or other massive changes in the genome that would prevent the formation of a viable organism[69].

3. TELOMERES AND AGING IN RODENTS

Rodent cells differ from those of longer-lived mammals. Human cells have relatively short telomeres compared to those of mice and rats (although not all rodents have long telomeres) [70-72]. Whereas most somatic human tissues and cells are telomerase negative, many mouse and rat tissues and cells are telomerase positive [72]. Although rodent cells undergo replicative arrest or slowed growth after a limited period of proliferation in culture, this is not a telomere-based senescence process. In mouse cells, the arrest is caused by oxidative damage resulting from the exposure of cells to 20% O_2, the usual conditions under which cells are cultured [73]. Whereas the cause of the replicative arrest of mouse cells in culture is not the same as that for human cells, some of the biochemical features of the nonreplicating state in rodent cells are very similar to those of senescent human cells [74]. The function of p53, although not pRb, is required in mouse cells; the arrest is maintained by high levels of cell cycle inhibitory proteins; and SA-β-gal is induced. The frequency of escape from this period of slowed growth (spontaneous immortalization) is very high in mouse cells. This may be one manifestation of more general differences in the consequences of DNA damage between mouse and human cells, as reviewed below.

In wild type mice, cells have not been shown to undergo telomere shortening in tissues during aging. However, telomere shortening can be produced in mice by inactivation of the telomerase RNA gene (*Terc*$^{-/-}$ mice) [70]. After three generations, the normally long mouse telomeres have shortened in the *Terc*$^{-/-}$ animals, and these mice present a picture of a "segmental progeroid syndrome," i.e. some aspects of the phenotype

resemble accelerated aging. They have premature graying and loss of hair, poor wound healing, gastrointestinal defects, infertility, decreased adipose tissue, and a shortened life span[75-77].

Terc$^{-/-}$ mouse embryonic stem cells show progressive telomere shortening in culture that eventually results in growth arrest[78]. As telomeres shorten in these cells there is an increasing frequency of chromosome aberrations. Like wild-type mouse cells, *Terc*$^{-/-}$ fibroblasts undergo a high rate of spontaneous immortalization, but in this case this is via the ALT pathway[79]. These observations lead to the tentative conclusion that the short-telomere checkpoint that leads to G1 arrest (i.e. replicative senescence or M1) is lacking in mouse cells. The chromosomal abnormalities seen in *Terc*$^{-/-}$ mouse cells as telomeres shorten, leading eventually to impaired cell division, indicate that the growth arrest is a form of crisis/M2 rather than replicative senescence. The important distinction is that cell cycle arrest in *Terc*$^{-/-}$ mouse cells appears to be directly related to chromosomal dysfunction, whereas human cells arrest in G1 at a telomere length that is still much longer than the length seen in human cells that have bypassed M1 and are in M2/crisis.

These differences in the behavior of human and mouse cells may be examples of a more general species difference in the late consequences of DNA damage [80]. Human cells may have a more efficient arrest following chromosome damage than mouse cells. In cells that survive an insult that causes DNA damage, the damage may become fixed in the form of mutations and chromosome aberrations. When cells with chromosome damage continue clonal expansion, there has been a failure of the checkpoints that would normally eliminate such cells by apoptosis or senescence.

In a population of irradiated human cells, many clones with chromosome aberrations disappear in the first few divisions after radiation exposure [81]. On the other hand, the frequent spontaneous immortalization of primary mouse cell cultures, with the production of cell lines that are often aneuploid [82], may be an example of the failure of a checkpoint that should eliminate such cells by apoptosis or senescence.

There is clearly a close relationship between the checkpoints that operate to eliminate cells with chromosome damage and the replicative senescence checkpoint, in the form in which it operates in human cells [83]. Telomere-based replicative senescence itself, as it occurs in human cells, could in fact be an example of the difference in the reaction of human and mouse cells to chromosome damage. As reviewed above, most evidence suggests that both human and bovine fibroblasts, cells that show telomere-based replicative senescence, arrive at senescence with a near-normal karyotype. However, it is possible that the last cell division that takes place before one or both daughter cells become terminally senescent creates a

chromosome fusion or break that triggers senescent growth arrest [83]. If so, arrest would appear to occur efficiently in human cells, whereas in mouse cells chromosome aberrations that should cause growth arrest often fail to do so and become stably propagated in descendant cells. The basis for these differences requires more study [80].

4. THE FUNCTION (EVOLUTIONARILY CONSERVED SELECTIVE VALUE) OF REPLICATIVE SENESCENCE: AN ANTI-CANCER MECHANISM

Because TERT appears to be re-expressed in the majority of human cancers [13], it is been hypothesized that the process by which TERT is repressed in most somatic cells is an anti-cancer mechanism. The best evidence that TERT repression is indeed an anti-cancer mechanism in human cells comes from data showing that the well-known oncoproteins Ras and SV40 T antigen cannot transform a normal human cell into a tumor cell unless they are also expressed together with TERT[84]. Presumably, the reason that TERT can co-operate with other oncogenes is that, during the process by which a normal cell and its descendants become fully malignant tumor cells, many cell divisions must take place and telomeres would become critically short, unless the cell activates telomerase or other mechanisms to prevent telomere shortening.

Thus the combination of initially short telomeres, suppression of TERT expression, and a checkpoint that triggers replicative senescence in response to short telomeres, together provide an anti-cancer mechanism. The existence of this anti-cancer mechanism in humans might contribute to the large difference in susceptibility to cancer (calculated on a per cell basis) between mice and humans. Suppose that mice and humans have the same risk of dying of cancer over their life spans (approximately true at least for some strains of mice [85]. However, a human being is about 3,000 times heavier than a 25-gram mouse and lives about 30 times as long. Consider also that cells are about the same size in mice in humans and that cell turnover occurs at about same rate. All these assumptions may not be entirely correct but this does not substantially affect the basic validity of this argument. Then it is evident that human cells are approximately 90,000 times more resistant to tumorigenic conversion per unit of time than are mouse cells. Presumably, as part of the evolution of the life history of the human species, anti-cancer mechanisms evolved that were not present in short-lived ancestors. In this case the anti-cancer process may provide an example of antagonistic pleiotropy, the genetic event (repression of TERT) having

beneficial effects in early life span and possibly negative effects in late life span[86,87].

In mice, such anti-cancer strategies are unnecessary for their life history. Their small size and short life span means that they are not more likely than humans to die of cancer before being able to reproduce. Thus there has not been an evolutionary selective pressure to repress TERT expression in this species (and presumably in other similar small short-lived mammals, although this has not yet been well studied). Presumably there are similar arguments that can be made in terms of trade-offs between the advantages and disadvantages of long and short telomeres [74]. Evidently, however, an organism that adopts TERT repression as an evolutionary anti-cancer strategy must also have short telomeres, or TERT repression becomes irrelevant to suppression of malignant transformation.

If suppression of TERT/short telomeres is a strategy that has evolved as an anti-cancer mechanism we are left with a puzzle. The senescent state appears to be a universal process, present in both human and mouse cells that is a reaction to certain kinds of DNA damage. The kinds of damage that cause cells to enter this state are very similar to those types of damage that cause other cells to enter apoptosis. From the point of view of the organism and the genome, making cells undergo apoptosis makes sense because the damaged cell and its progeny, carrying potentially damaged copies of the genome, are removed from the body. One may consider cells to be very cheap in terms of the overall economy of the body – millions of cells are born and die every day and there would seem to be no reason why cells should be preserved via the "replicative senescence" process, rather than killed off via apoptosis.

Therefore, one must ask the question, does replicative senescence occur in tissues in vivo? There is much evidence that telomere shortening occurs in tissues, but very little evidence directly addresses the question of whether telomere shortening causes cells to reach the same state in the body as it does in cell culture. In a recent review, Hanahan and Weinberg state: "The above-cited observations [on replicative senescence] might argue that senescence, much like apoptosis, reflects a protective mechanism that can be activated by shortened telomeres or conflicting growth signals that forces aberrant cells irreversibly into a G0-like state, thereby rendering them incapable of further proliferation. If so, circumvention of senescence in vivo may indeed represent an essential step in tumor progression that is required for the subsequent approach to and breaching of the crisis barrier. But we consider an alternative model equally plausible: senescence could be an artifact of cell culture that does not reflect a phenotype of cells within living tissues and does not represent an impediment to tumor progression in vivo. *Resolution of this quandary will be critical to completely understand the acquisition of limitless replicative potential."* [emphasis added] [88].

To state the problem in another way, the short telomere/TERT repression combination is generally accepted to be an anti-cancer mechanism, but we do not know if the anti-cancer effect is mediated through the replicative senescence/M1 cell cycle block, as it is observed in cell culture.

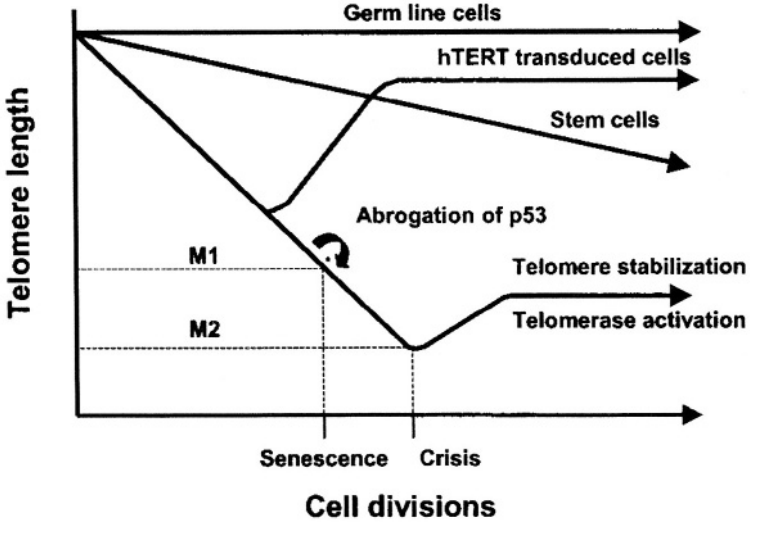
5. BY WHAT MECHANISMS CAN REPLICATIVE SENESCENCE ACT TO SUPPRESS CANCER?

As reviewed above, and more extensively elsewhere [89], there is considerable evidence that telomere shortening occurs in tissues in vivo and one might expect therefore that short-telomere cells in tissues would stop dividing when they reach the M1 telomere length. However, observations made in the human genetic disease dyskeratosis congenita (DKC) suggest that perhaps this does not occur in vivo. DKC is a disease of impaired telomerase activity and shortened telomeres [90-92]. In one form of the disease (X-linked) the DKC1 gene is defective; its protein product, dyskerin, is required for proper RNA processing, including the RNA of the telomerase ribonucleoprotein complex. In an autosomal dominant form of DKC, telomerase RNA is mutated. In these syndromes there are proliferative defects in tissues known to have telomerase-positive stem cells (hematopoietic system and skin). DKC patients have very short telomeres in fibroblasts and white blood cells. They usually die of bone marrow failure at a young age. However, the disease is also associated with chromosomal abnormalities and early death from some cancers. Whether replicative senescence accounts for some of the pathology in DKC is unknown, but the chromosomal instability and increased cancer suggest that shortening telomeres in human tissues in vivo might lead to crisis rather than replicative senescence.

If so, to some extent this resembles the situation in *Terc*$^{-/-}$ mice [70], where chromosomal aberrations cause defects in proliferation. As reviewed above, these mice lack telomerase activity and undergo generation-dependent telomere shortening. Moreover cells from these mice lack an M1/replicative senescence arrest in culture. They also appear to lack this form of growth arrest in vivo. *Terc*$^{-/-}$ mice are viable to the sixth generation, when infertility prevents a seventh generation. Well before this point, increased numbers of end-to-end chromosome fusions are observed (0/metaphase in wild type; 0.26 in generation 2; 0.56 in generation 4; and 1.93 in generation 6) [70]. Regeneration of the liver after partial hepatectomy is impaired in G6 *Terc*$^{-/-}$ mice [93]. Flow cytometry shows that many hepatocytes have a 4N DNA content. Thus cells do not arrest in G1, as expected if the short telomeres trigger replicative senescence, but instead hepatocytes have impaired

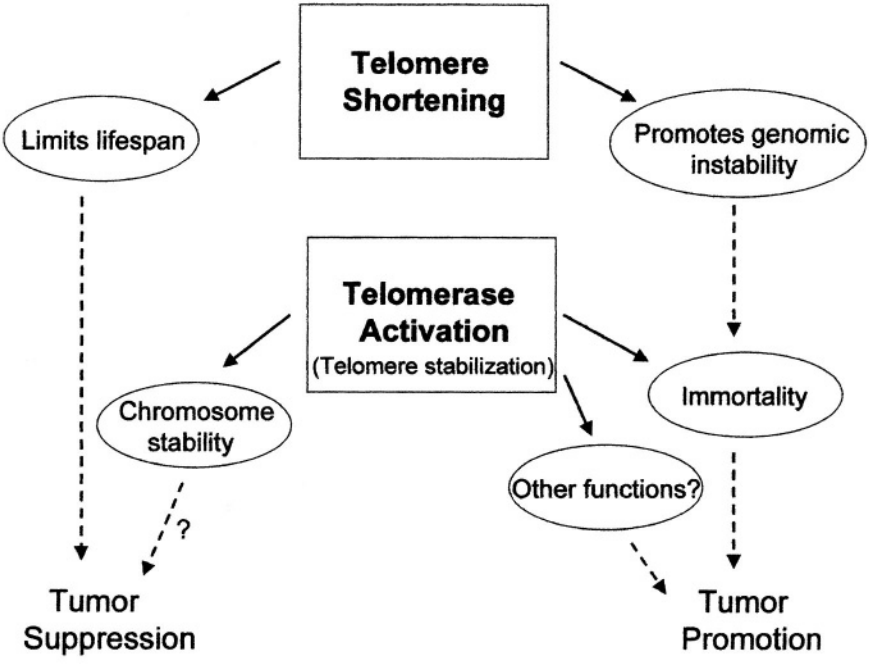
progress through mitosis. Many aberrant mitotic figures are observed. In G6 *Terc*$^{-/-}$ mice, excess apoptosis of germ cells is observed, providing an explanation for infertility. Apoptosis was blocked, and fertility restored, when G6 *Terc*$^{-/-}$ mice were generated in a p53$^{-/-}$ background [94]. Therefore, at least in germ cells, short telomeres appear to trigger p53-dependent

Figure 1. Replicative senescence and crisis. In germ cells telomere length is maintained by telomerase, but most human somatic cells do not have sufficient telomerase activity to maintain telomere length and undergo telomere shortening with each cell division. Stem cells have telomerase activity but may not maintain full telomere length. When telomeres reach a certain length (point "M1") they enter replicative senescence. The expression of oncoproteins such as SV40 T antigen enables cells to bypass the point by inactivation of pRB/p16 or p53. Such cells continue to undergo telomere shortening to the "M2" point, or crisis. Rare cells in crisis cultures activate telomerase by unknown mechanisms and thereby are able to grow indefinitely with a stabilized telomere length. When cells are cultured in adequate conditions, ectopic expression of hTERT allows cells maintain telomere length greater than the M1 length. Reproduced with permission from Cong et al., 2002.



apoptosis. When this block is bypassed in the $p53^{-/-}$ background, two more generations of $Terc^{-/-}$ mice are possible before a non-p53 dependent "genetic catastrophe" occurs [94].

Figure 2. The "telomere paradox": short telomeres may both suppress cancer formation and promote cancer formation. Reproduced with permission Masutomi and Hahn, 2003.



Late generation $Terc^{-/-}$ mice with short telomeres also show increased cancer incidence [73], as do human DKC patients. However, when G6 $Terc^{-/-}$ were generated on the cancer-prone $INK4A^{-/-}$ background cancer incidence was reduced in comparison with telomerase-positive $INK4A^{-/-}$ mice [95].

Thus, the data point to a telomere paradox (Figure 2). If short telomeres/repression of TERT is an anti-cancer mechanism, why does a disease in which these traits are exaggerated display an increased cancer

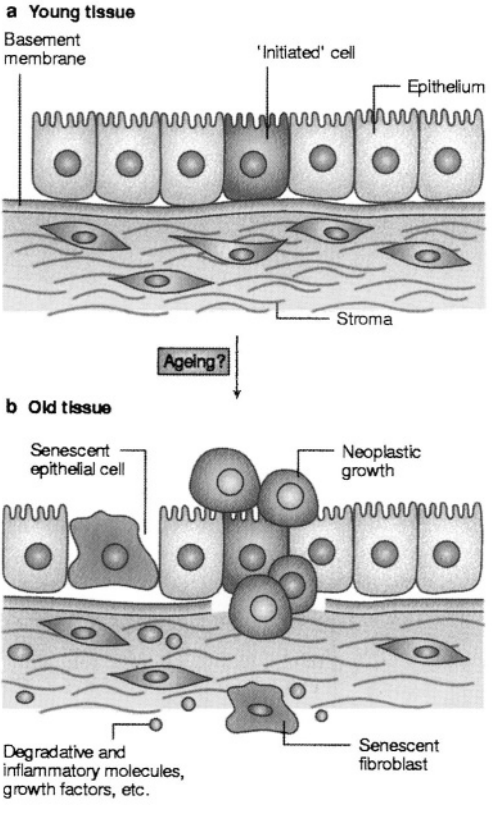
risk? This paradox currently lacks a satisfactory explanation. As it is evident that humans as a species have a greater need for anti-cancer mechanisms than rodents, the conclusion that short telomeres/repression of TERT is an anti-cancer mechanism seems inevitable, yet the means by which the anti-cancer effects are exerted remain unresolved.

6. CAN REPLICATIVE SENESCENCE ACT TO PROMOTE CANCER?

If cells that reach the M1 telomere length truly "senesce" in vivo, and then undergo the same kinds of changes in gene expression as they do in culture, this process could certainly have adverse effects on tissue function. In this regard one can pose a series of related questions: (i) Do cells undergo telomere shortening to the extent of reaching the M1 telomere length? (ii) If so, is the consequence of this the same as it is in culture, i.e. the generation of senescent cells, or do they suffer some other fate (e.g. crisis)? (iii) If they become senescent, do such cells accumulate in tissues, or are they eliminated by some part of either the acquired or innate immune system? (iv) If they do accumulate in tissues, do they exert a pro-carcinogenic effect because they secrete proteases and cytokines?

The most significant evidence for the occurrence of senescent cells in aging tissues is the occurrence of cells that stain for senescence-associated β-galactosidase (SA-β-gal) in tissues as a function of age. The presence of SA-β-gal$^+$ cells was first reported for human skin [32] and was subsequently shown in the rhesus monkey in retinal pigmented epithelium [96] and in the epidermis [97]. In these studies the number of SA-β-gal$^+$ cells increased as a function of donor age. These intriguing observations raise several questions. First, we do not know the mechanism by which such cells are formed; if their existence has consequences for tissue function, the mode by which they become senescent should be understood, so that appropriate interventions (both experimental and clinical) can be designed and tested. Second, whether such cells in vivo actually have the same range of changes in gene expression observed in replicative senescent cells in culture is also unknown. This is important, because it has been speculated that these changes may result in a pro-carcinogenic state in tissues that could aid the growth of pre-malignant cells and provide a permissive environment for tumor progression [98, 99] (Figure 3). It is conceivable that many properties of aging tissues, including an increased rate of neoplastic conversion, might result from the presence of relatively small numbers of replicative senescent cells.

Figure 3. Hypothetical scheme by which the presence of senescent cells in tissue during aging could exert a pro-neoplastic effect. In the young tissue, a cell with potentially oncogenic mutations ("initiated") is prevented from uncontrolled growth by neighboring cells. In the old tissue, senescent cells secrete enzymes and cytokines that enable the initiated cell to grow into a cancer. Reproduced with permission from Campisi, 2003.



Many more studies are needed in this area. First, the variety of tissues and the range of donor ages that have been surveyed so far is very small, and it is not possible yet to determine whether the occurrence of SA-β-gal$^+$ cells is an inevitable part of normal aging or alternatively evidence of a pathological process. Studies in the prostate, liver, and vascular endothelium are suggestive of an accumulation of SA-β-gal$^+$ cells in disease states [100-102]. Second, more studies are needed to show whether SA-β-gal$^+$ cells are generated by telomere shortening or by some other process. The suspicion that in some cases telomere shortening is not involved exists for retinal pigmented epithelial cells, because these cells are mostly postmitotic in adult life [103]. These cells may have entered the senescent state as a form of stress-induced senescence as a result of exposure to oxidative damage.

Conversely, more studies are needed to show the fate of cells with shortening telomeres in tissues. One consequence of our lack of knowledge in this area is that SA-β-gal$^+$ staining cannot be used as an *in situ* assay for cells that have exhausted their replicative capacity in vivo by telomere shortening.

One final point is there has not yet been enough consideration given to alternate fates of cells that have undergone telomere shortening in vivo and whose telomeres have reached the M1/senescent length. The possibility should be considered that their changes in gene expression might become so marked that they are no longer recognized as "self" and are eliminated by either acquired or innate immune functions, much as incipient cancer cells are eliminated by immune surveillance [104].

5. CONCLUSIONS

Replicative senescence appears to be a form of protection against cancer, but the means by which it may have this action are not yet clear. More research is needed to understand the fate of cells with short telomeres in tissues during aging, to understand the effects on neoplasia of telomere shortening in tissues in vivo, to understand whether telomere shortening results in the accumulation of senescent cells in tissues, and, if so, what effects this may have on tissue function.

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Chapter 4

THE INFLUENCE OF ADVANCED AGE ON CANCER OCCURRENCE AND GROWTH

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Recently there has been an increased awareness of the overlapping biologic pathways operant in the processes of aging and carcinogenesis. Coincidently, there has been increased interest in both basic and clinical research pertaining to cancer and aging. There remain many unanswered questions about cancer management in geriatric patients, in part, due to the lack of understanding of the influence of age on tumor biology. This review will attempt to establish a framework around themes in aging biology that are relevant to the development and progression of cancer.

1. NORMAL AGING

It is a central gerontologic principle that aging is not a disease. The functional declines that accompany normal aging have been well characterized[1], but under normal circumstances do not account for symptoms or disease. For example, kidney function declines with age [2], and, in fact, has proven to be a useful biological marker of aging (see below). Yet, clinical consequences of this change in renal function, in the absence of a disease or the exposure to an exogenous nephrotoxic agent, do not occur commonly. Similarly, the bone marrow changes with age (discussed elsewhere in this volume).

Aging is not a disease but the consequences of aging may make an individual susceptible to disease. For example, changes in the immune system may render an individual susceptible to reactivation of tuberculosis [3,4]

or herpes zoster[5] and less capable of responding to influenza vaccine with protective titers of antibody [6-8]. The immune decline, however, is not of sufficient magnitude or duration to account for the increased incidence of cancer in old people [9]. In fact, based upon findings in experimental animals, it has been postulated that immune senescence may contribute to the observed reduced tumor growth and spread in a variety of tumors (discussed below).

2. LIFESPAN AND MAXIMUM SURVIVAL

From the perspective of those who study aging, there is an important distinction made between median (life expectancy) and maximum life span. Over the past several decades, with the advent of modern sanitation, refrigeration and other public health measures including vaccination and antibiotics, there has been a dramatic increase in median survival [10]. Early deaths have been diminished and more individuals are reaching old age. In the United States today, life expectancy now approaches 80 years [11]. Median survival is what concerns public health officials and health care providers but for those studying the biology of aging, it is maximum survival that is the focus of greatest attention. It is worthwhile to note that it has been estimated that if atherosclerosis and cancer were eliminated from the population as a cause of death, about ten years would be added to the average life span, yet there would be no change in maximum life span [12].

The oldest human being alive today is approximately 120 years old. What is intriguing is that the record has remained stable, unchanged by the public health initiatives mentioned above. In fact, there has been some recent data presented that the maximum survival is actually declining in the United States [13,14]. In the laboratory, similar limits have been established for a variety of species. Drosophila, free of predators, can live 30 days, whereas C57BL/6 mice in a laboratory environment and allowed to eat a healthy diet ad libitum, may survive 40 months. What is interesting is that, unlike the public health initiatives in humans, experimental interventions in lower species have been associated with a prolongation of maximum survival. In Drosophila, for example, transgenic offspring producing extra copies of the free radical scavenging enzymes superoxide dismutase and catalase survived about 33% longer than controls [15]. However, there has been some criticism of this work based on the claim that the controls were unusually short lived. In mammalian species, the only experimental intervention that characteristically prolongs maximum survival is the restriction of caloric intake. In fact, dietary restriction (DR) has become a common experimental paradigm exploited in the investigation of primary processes of aging (for a review see reference 16).

3. CELLULAR VERSUS ORGANISMAL AGING

There has been much written about cellular senescence and the events that lead up to cell death (reviewed in reference 17). After a finite number of divisions, normal somatic cells invariably enter a state of irreversibly arrested growth, a process termed replicative senescence [18]. In fact, it has been proposed that escape from the regulators of senescence is the antecedent of malignant transformation. However, the role of replicative senescence as an explanation of organismal aging remains the subject of vigorous debate. The controversy relates, in part, to the fact that certain organisms (e.g., Drosophila, C. elegans) undergo an aging process, yet all of their adult cells are post replicative.

What is clear is that the loss of proliferative capacity of human cells in culture is intrinsic to the cells and not dependent on environmental factors or even culture conditions [18]. Unless transformation occurs, cells age with each successive division. The number of divisions turns out to be more important than the actual amount of time passed. Thus, cells held in a quiescent state for months, when allowed back into a proliferative environment, will continue approximately the same number of divisions as those that were allowed to proliferate without a quiescent period [19].

The question remains whether this in vitro phenomenon is relevant to animal aging. One suggestive observation is that of fibroblasts cultured from samples of old skin undergoes fewer cycles of replication than those from young [20]. Furthermore, when various species are compared, replicative potential is directly and significantly related to life span [21]. An unusual β-galactosidase with activity peaks at pH6 has proved to be a useful biomarker of in vitro senescence because it is expressed by senescent but not presenescent or quiescent fibroblasts [22]. This particular β-galactosidase isoform was found to have the predicted pattern of expression in skin from young and old donors with measurably increased levels in dermal fibroblasts and epidermal keratinocytes with advancing age [22]. The nature of the expression of this in vivo biomarker of aging in other tissues will be important to discern.

4. IMMUNITY AND AGING

There is a well-characterized deficit in immune function with advancing age, but the consequences are not fully established. It is apparent that otherwise healthy older individuals are more susceptible to reactivation of tuberculosis [3,4] or Herpes zoster [5], and responses to vaccines, such as the commercially available and widely used influenza hemagglutinin, are lower [23-25]. However, it has been postulated that other age-associated diseases,

such as cancer [26], atherosclerosis [27,28], diabetes [29], and even Alzheimer's disease [30,31] have been related to the immune decline with age. Yet more recent evidence would argue that inflammation may contribute to Alzheimer's disease (see below).

What can be said with confidence is that there are changes in T cell function with age that result in decreased proliferation when measured in vitro [32]. When studied as a population, there appears to be an accumulation of T cells with cell surface characteristics of memory cells, whereas in contrast, there is a relative decrease in naive T cells [33]. B cell function, including the capacity to make antibody remains intact, although certain intrinsic alterations have been noted [34]. Immunoregulatory functions are affected by the aging process and paraproteinemia, and autoantibody is observed with increasing frequency with each advancing decade. In general, the paraproteinemia is an indicator of dysregulated immunity, but it is considered not to be the antecedent of multiple myeloma [35-37]. However, myeloma does increase in incidence in geriatric populations and it must be distinguished from the benign paraproteinemia of aging. Typically, this is accomplished by examination of bone marrow, skeletal x-rays and renal function and serial (e.g., every 3 months) determination of paraprotein level [38].

Another indication of dysregulated immune function is the alterations in certain key cytokines, measured in plasma, culture supernatants, or in the appropriate tissue microenvironment. Notably and consistently interleukin-2 (IL-2) levels and function decrease with age [39], and IL-6 levels increase [40]. The decline in IL-2 may account for a significant component of the measured decline in T cell function and the increased IL-6 has been implicated in the pathogenesis of certain age-associated diseases (osteoporosis, Alzheimer's disease and cancer and the syndrome of frailty [41,42]).

5. IMMUNESENESCENCE AND CANCER

Proponents of an immune explanation point to experiments in which outbred strains of mice with heterogeneous immune functions were followed for their life span [45]. Those who demonstrated better functions early in life (as determined by a limited panel of assays available at the time on a small sample of blood) were found to have fewer spontaneous malignancies and a longer life than those estimated to be less immunologically competent. Recently, a report from Japan [46] in which a cohort of individuals, on whom lymphocyte (specifically NK cell function) measures were obtained decades earlier, demonstrated a reduced incidence of cancer in those who had better

lymphocyte functions. These and other similar observations do support the notion of immune surveillance and indicate the potential importance of immunesenescence in explaining the great rise in incidence of cancer with age.

Despite the controversy regarding the importance of immune surveillance, there is much greater consensus on the importance of the immunodeficiency of aging in the clinical management of cancer, eluding the problems associated with infection and disease progression.

6. THE AGING HOST AND THE DEVELOPMENT OF CANCER

Carcinogenesis is a multistage process involving serial alterations of cellular genes. These include oncogenes and antiproliferative genes (antioncogenes), which modulate cell proliferation and genes which prevent apoptosis (programmed cell death). It is now understood that oncogenes encode proteins with a myriad of functions including growth factors, growth factor receptors, enzymes involved in the transduction of proliferative signals, DNA synthesis and replication (for a review, see reference 47). Similarly, antioncogenes encode cell proliferation or DNA-replication inhibiting proteins and apoptosis-preventing genes encode proteins that inhibit the activation of endonucleases which would otherwise disrupt the template function of DNA and result in cell death [48].

The multistage nature of carcinogenesis has been demonstrated in experimental models with strong circumstantial support in human cancers. For example, for the case of colorectal cancer, Vogelstein and colleagues [49] described a sequence of genetic alterations leading from normal mucosal epithelium to invasive carcinoma. One step, the loss of the Familial Adenomatous Polyposis (FAP) gene on the 5th chromosome, is associated with hyperproliferation of the mucosal cells and formation of adenomatous polyps. Additional changes in the expression of the p53 gene on chromosome 18 and the DCC gene on chromosome 17 may lead to a more malignant phenotype. Likewise, in the case of brain tumors, loss of a portion of the 17th chromosome (17p) is seen in malignancy of all grades, whereas loss of chromosome 10 and of the genes encoding interferon receptors was found only in glioblastoma multiforme [50]. These changes may provide the genetic basis for the transformation from indolent to more aggressive disease. Sequential genetic changes leading to more aggressive neoplasms have been reported in many other diseases, including breast, cervical, renal and lung cancer [51-57].

6.1 Serial Stochastic Events

The interpretation of carcinogenesis as a multistage process presents at least two non-mutually exclusive explanations for the increasing incidence of cancer with age. The first and simplest is that the tissues of an older person will have, over time, sustained the serial stochastic events involved in carcinogenesis. Accordingly, the cancers more prevalent among the aged, such as prostate, colon or breast cancer, are those involving a greater number of steps. In contrast, this hypothesis would predict that tumors more common in young people (lymphoma, leukemia, neuroblastoma, etc.) would require fewer steps in the progression from normal to the malignant state.

6.2 Age as a Risk Factor

The second hypothesis holds that age itself is a risk factor for cancer because the process of aging involves genetic events that are similar to those occurring during early carcinogenesis. Thus, the number of cells that would be susceptible to the effects of late-stage carcinogens increases with age. Both experimental and clinical evidence support this theory. Cytogenetic and molecular changes observed in early carcinogenesis are also seen in cells maintained in long-term culture. These changes include formation of DNA adducts, DNA hypomethylation, chromosomal breakage and translocation [58,59]. Also, the accumulation of iron commonly observed in some aging cells, may cause oncogene activation and antioncogene suppression [59-61]. The likelihood of neoplastic transformation after exposure to late-stage carcinogens is higher in tissues from older animals than in those of younger animals, both in tissue culture and in cross-transplant experiments [62-65].

Epidemiological data for some cancers suggest that the susceptibility to late-stage carcinogens increases with age [66]. The comparison between the incidence of melanoma and of squamous cell carcinoma (SCC) of the skin is particularly illustrative [67,68]. Whereas, in the United States the incidence of melanoma plateaus at age 45 for women and 61 for men, the incidence of SCC continues to rise even beyond age 85. This is what might be predicted if there were more steps in the generation of SCC than in melanoma. However, the increased number of steps is not the total explanation because the incidence of SCC increases logarithmically with age [68]. This suggests either the association of longevity with a genetic predisposition to SCC (unlikely) or, the increased susceptibility with age to late-stage carcinogens. It should be underscored that both basic and clinical data suggest that there is an increased susceptibility and it may be tissue and organ specific. For example, skin epithelium, liver and lymphoid tissues, but not nervous or muscular tissues, show increased susceptibility to late-stage carcinogens in older rodents [69]. Similarly, the incidence of melanoma and mesothelioma in

humans demonstrates an age-related plateau, suggesting that these tissues are not more susceptible to late-stage carcinogens [66-68].

Other age-related factors that may increase the risk of cancer include reduced DNA repairing ability and decreased carcinogen catabolism [70]. It has been proposed that these lead to an accelerated carcinogenic process with more rapid generation of cells susceptible to late-stage carcinogens (promoters)[71].

7. TUMOR AGGRESSIVENESS IN THE AGING HOST

There has been a long-held but incompletely documented clinical notion that cancers in older people are "less aggressive" (Table 1). However, epidemiological data from tumor registries or large clinical trials have not been supportive. This may be because this type of data is confounded by special problems common to geriatric populations (e.g., comorbidity, "poly-pharmacy," physician or family bias regarding diagnosis and treatment in the elderly, and age-associated life stresses) and these factors may counter any primary influence that aging might have on tumor aggressiveness. However, there is experimental support for the contention that there is reduced tumor aggressiveness with age. Data obtained from laboratory animals with a wide range of tumors under highly controlled circumstances demonstrate slower tumor growth, fewer experimental metastases, and longer survival in old mice[72-75].

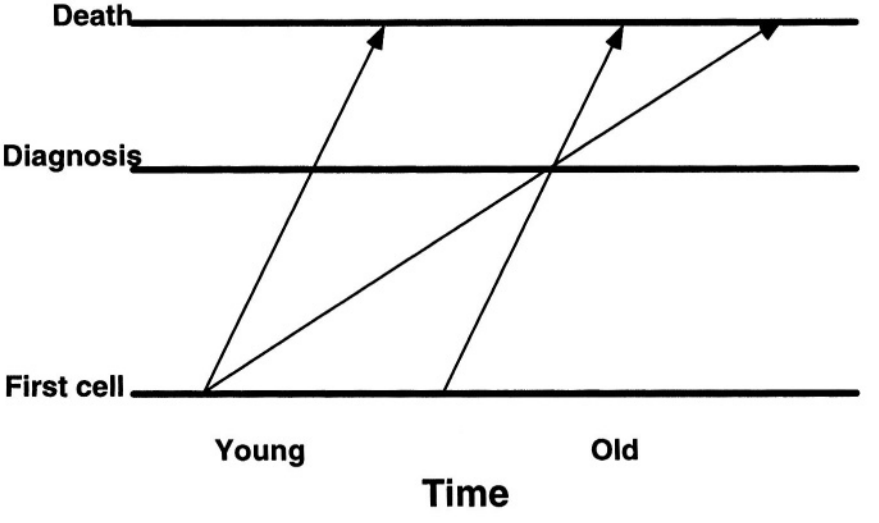
What accounts for the age-associated changes observed in these experimental systems? One explanation derives from the understanding that the tumors, although histologically quite similar, may be biologically very different in old patients. For example, breast cancer cells are more likely to contain estrogen receptors, and leukemic cells have cytogenetic abnormalities in elderly patients with those disorders. Each of these

Table 1. Cancers Reported to Have Reduced or Altered Patterns of Growth with Age

Breast
Colon
Lung
Prostate
Renal

Tumors originating in these organs have been reported to have reduced tumor growth rates or longer survival in older patients. For a review of these reports, see reference 75.

Figure 1. One explanation for varying tumor aggressiveness with age. Rates of tumor proliferation may play a role in the apparent slower growth of tumors. For example, if two tumors, one fast growing and one slow both arise at the same stage of life, the faster growing tumor would present clinically at a younger age. This model might explain why tumors arising in younger patients tend to be more aggressive, and why there is such great heterogeneity in tumor characteristics (such as aggressiveness) in older individuals.



associations has prognostic significance. Furthermore, there is the issue of the "time line" artifact (Figure 1) that implies that old patients (more so than young) may develop slow growing tumors on the basis of time required to develop such slow tumors. Such is, of course, consistent with the multistep hypothesis as discussed above.

It is probable that certain factors that influence tumor growth change with age. With this in mind, various endocrine, nutritional, wound healing, and angiogenesis factors have been explored. For some tumors, age-associated changes in these factors have been correlated with reduced tumor growth [76-80]. However, several early observations led to the seemingly paradoxical conclusion that immune senescence accounted for a large component of the observed reduced tumor growth with age. For example, B16 melanoma grows less well in congenitally immune deficient mice [81] and in young mice rendered T-cell deficient [119]. Furthermore, when young, thymectomized, lethally irradiated mice received bone marrow or splenocytes from old donor mice, tumor growth was less than when the spleen or bone marrow was from young donor mice[73,74].

It is believed that competent immune cells provide factors that augment tumor growth under certain circumstances. If a tumor is only weakly antigenic, non-specific growth stimulatory factors provided by lymphocytes or monocytes may actually counteract the inhibitory forces provided by those same cells (because of the lack of tumor antigen). In this situation immune deficiency does not render a host more susceptible to aggressive tumor growth and spread; in fact immune deficiency renders a host more resistant because those cells are less likely to provide the non-specific stimulatory factors. This hypothesis is akin to the immune enhancement theory promoted several decades ago by Prehn and colleagues [82]. Briefly stated in the context of cancer and aging; the positive growth, angiogenic and other tumor stimulatory signals produced nonspecifically by cells considered part of the immune system will be less by cells from old animals. In other words, the "soil" is less fertile for aggressive tumor growth

8. CONCLUSIONS

It has been said that all medical oncologists, with the exception of those who restrict their practice to pediatric patients, are 'geriatric oncologists'. This, of course, because the average age of cancer is in excess of 65 years and the median age of most common adult tumors approaches 70 years. Similarly, scientists studying the mechanisms of cancer development and growth are uncovering and elucidating some of the basic molecular and cellular processes of aging. These include the controls of cellular proliferation, mechanisms of DNA repair, and programmed cell death. There are striking voids in our understanding of the basic mechanisms of aging, but one can not help but have the sense that the advances in this field will have the added value of enhancing our understanding of tumor development and growth.

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Chapter 5

AGE AND CO-MORBIDITY IN CANCER PATIENTS: A POPULATION-BASED APPROACH

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The mean age of patients diagnosed with cancer is increasing in western countries due to rising incidence rates of most cancers with age and ageing of the population. In most European countries more than 40% of all new patients with cancer are over the age of 70, which implies that they increasingly suffer from one or more other serious (chronic) diseases and from interactions with and side effects from their treatment. Besides affecting the life expectancy co-morbid conditions and their treatment may complicate the clinical management of cancer patients, especially when they are frail. Since they are often excluded from clinical trials, little is known about treatment outcome, such as complications, quality of life and survival. Choice of curative treatment of cancer for older patients may be influenced by the physical condition of the patient (co-morbidity, reduced functional reserves, interaction between medications, performance status), the psychological condition (depression, dementia) and social parameters (informal care, mobility)[1-3].

This chapter focuses on the role of age and co-morbidity in cancer patients. The value of studying co-morbidity is demonstrated by data of the

population-based Eindhoven Cancer Registry [4]. We were looking for answers on questions on guideline adherence from local clinicians who increasingly experienced problems with an increasing number of elderly patients. The clinical context is one of community hospitals only, within the framework of the Comprehensive Cancer Centre [5]. We give insight in the prevalence of co-morbidity in unselected cancer patients, and the effects of co-morbidity on treatment and prognosis.

1. METHODS

The Eindhoven Cancer Registry records data on all patients newly diagnosed with cancer in the southern part of the Netherlands, an area with now 2.3 million inhabitants and only general hospitals. Since 1993 serious co-morbidity with prognostic impact has been recorded for all patients. The Charlson Co-morbidity Index is most widely used for recording co-morbidity and was validated in various studies [6]. We used a slightly adapted version of this index for recording co-morbidity (Table 1). Co-morbidity was defined as life-shortening diseases that were present at the time of cancer diagnosis and/or received some treatment or surveillance.

Table 1. Classification of co-morbidity, according to an adapted version of the list of Charlson et al. (1987)

Chronic Obstructive Pulmonary Diseases (COPD)
Cardiovascular disease: myocardial infarction, cardiac insufficiency, angina pectoris, CABG (coronary artery bypass graft)
Peripheral arterial disease: intermittent claudication, abdominal aneurysm, surgical intervention
Cerebrovascular diseases (cerebrovascular accident, hemiplegia)
Other malignancies (except basal cell skin carcinoma)
Hypertension
Diabetes mellitus
Other:
- autoimmune diseases: sarcoidosis, Wegener's disease, SLE (systemic lupus erythematosis)
- rheumatoid arthritis (only severe)
- kidney diseases: glomerulonephritis, pyelonephritis
- gastrointestinal: stomach ulcer & resection, colitis
- liver diseases: cirrhosis, hepatitis
- dementia
- chronic infection

The data were extracted from the medical records between 6 and 18 months after diagnosis, when the trained registry personnel does its routine view. Previous admissions, letters from and to general practitioners (every patient has his GP in the Netherlands) and other specialists, the medical history and preoperative screening were used as sources. On average, it takes about 5 minutes per patient to record co-morbidity. The medical record is generally regarded as the most complete source of information on the patient's past and current health status[7].

Patients with cancer of the esophagus, stomach, colon or rectum, pancreas, lung, breast, cervix uteri, corpus uteri, ovary, prostate, bladder, kidney, and non-Hodgkin's lymphoma, newly diagnosed between 1995 and 2001 (N=48,030), were included for this overview. Patients with cancer diagnosed at autopsy (N=447 or 1%) were excluded. Treatment was classified as surgery (resection), radiotherapy, chemotherapy, hormonal therapy (or combinations) and 'other or none'. Surgery did not comprise diagnostic operations.

Survival analyses were restricted to patients with cancer of the colon or rectum, lung, breast, prostate or non-Hodgkin's lymphoma diagnosed between 1995 and 1999 (N=21,984). Vital status was available up to 1 April 2002. In addition to passive follow-up via the hospitals, this information was also obtained from the municipal registries in the area of the Eindhoven Cancer Registry and the Central Bureau for Genealogy. The latter is an institution that collects data on all deceased Dutch citizens via the civil municipal registries. In this way, information on patients who moved outside the registry area was also obtained. Patients who died outside the Netherlands were lost-to follow-up. The estimated proportion of these patients was less than 1%.

Survival time was defined as the time from diagnosis to death or the end of the study. Survival generally decreases with age, because other causes-of-death also take their share. The prevalence of co-morbidity increases with age. Therefore, we calculated relative survival rates which are an estimation of disease-specific survival. Survival of cancer patients is adjusted for mortality from all causes of death in the background population with the same age structure. Relative survival is calculated as the ratio of the observed to the expected rates [8]. Expected survival rates were estimated from life tables for regional male and female populations.

2. RESULTS

2.1 Prevalence

The prevalence of co-morbidity usually increased with age (Table 2), but remained stable or decreased above age 80 for some tumours. About 60% of all new cancer patients older than 65 also suffered from at least one other serious disease. The most frequent concomitant diseases were previous cancers, heart disease, hypertension, COPD, and diabetes mellitus, with prevalence rates up to 20%, 23%, 26%, 17%, and 16%, respectively. The prevalence of co-morbidity was highest for patients with lung cancer (over 70% for men aged 65 or older) and lowest for patients with breast cancer (about 55% for women aged 65 or older) (Table 3). The prevalence of cardiovascular diseases was higher among men compared to women, and was up to 35% of male and 28% of female patients with cancer of the digestive tract, lung, and kidney, and non-Hodgkin's lymphoma (Table 4). The prevalence of COPD was relatively high among older patients with lung cancer (31% of males and 24% of females), and also among men with esophageal and bladder cancer (20%). The prevalence of hypertension was highest among women with gynaecological tumors (38%) or adenocarcinoma of the kidney (up to 45%). High prevalence rates of diabetes in older patients were observed for cancer of the pancreas (up to 27%), cervix uteri (32%), corpus uteri (25%) and kidney (22%). The prevalence of diabetes in women with cervical cancer was twice as high among those with squamous cell than with adenocarcinoma, being 16% and 7% respectively (not shown).

The higher prevalence rates of digestive tract conditions among males compared to females were largely due to concomitant stomach ulcers (3.5% versus almost 2%) and previous gastrectomy (2.7% versus 0.6%), whereas the prevalence of colitis was about similar with 0.3%.

2.2 Treatment (see overview in Table 5)

Patients with colon cancer underwent surgery regardless of age or the number of co-morbid conditions: more than 95% with Dukes A-C did so and about 75% of patients with Dukes D. However, patients with Dukes C received less adjuvant chemotherapy with the rise of age: from 65% of patients at middle age to 33% of patients aged 65 or older (data 1997-2001). The proportion of patients with Dukes D receiving adjuvant chemotherapy decreased from 56% at middle age to 20% of patients aged 65 or older. The proportion receiving adjuvant chemotherapy also decreased from 27% of patients without co-morbidity to 17% of those with co-morbidity. The proportion of rectal cancer patients receiving adjuvant radiotherapy

decreased from 53% of patients younger than 65 to 37% of patients aged 65 or older, and also decreased with co-morbidity from 51% of patients without co-morbidity to 39% in case of co-morbidity.

Table 2. Age-specific prevalence (%) of serious concomitant diseases among newly diagnosed cancer patients in southeastern Netherlands, 1995-2001.

	MEN			WOMEN		
	Age (years)			Age (years)		
Concomitant disease	50-64 (n = 8294)	65-79 (n = 14,593)	80+ (n = 3362)	50-64 (n = 8210)	65-79 (n = 9621)	80+ (n = 3623)
Other cancers[a]	8.2	15	20	8.3	13	16
Heart disease	11	22	23	3.6	12	19
Peripheral vascular disease	4.4	8.8	7.4	1.4	3.1	3.2
COPD	9.5	17.1	17	5.7	8.4	7.1
Hypertension	12	16.2	12	14	26	24
Diabetes mellitus	5.7	9.3	11	5.2	14	16
Cerebrovascular disease	2.3	5.7	6.7	1.2	4.3	7.1
Autoimmune	0.7	1.0	0.9	0.9	1.6	1.8
Chronic infection	1.3	2.3	1.7	1.0	2.1	1.9
Central nervous system	0.1	0.5	2.5	-	0.8	4.1
Liver disease	0.9	0.7	0.5	0.5	0.5	0.3
Urinary	0.5	0.5	0.5	0.3	0.4	0.4
Gastro-intestinal	4.7	6.4	6.0	1.8	2.5	3.9
Connective tissue	0.3	0.1	-	0.3	0.2	0.3
Together[b]	**43**	**63**	**64**	**34**	**56**	**63**

Source: Eindhoven Cancer Registry; [a] also in same organ (but not metastasis of primary tumor), excluding basal cell skin Carcinoma; [b] more conditions per patient

Table 3. Age-specific prevalence (%) of the number of serious concomitant diseases among newly diagnosed patients with 13 major cancers in southeastern Netherlands, 1995-2001 Source: Eindhoven Cancer Registry.

				number of co-morbid conditions			
		age		None	1	≥2	unknown
tumor site	sex	(years)	N	%	%	%	%
All cancers	Men	50-64	8294	42	28	15	15
		65-79	14593	25	33	30	12
		80+	3362	20	32	33	16
	Women	50-64	8170	52	25	9	14
		65-79	9587	33	31	24	11
		80+	3618	23	31	32	14
Esophagus	Men	50-64	200	42	28	23	8
		65-79	216	24	33	39	4
		80+	57	21	35	37	7
	Women	50-64	73	48	30	11	11
		65-79	100	40	30	24	6
		80+	50	18	42	34	6
Stomach	Men	50-64	356	41	30	18	11
		65-79	619	26	32	34	7
		80+	171	18	32	40	10
	Women	50-64	132	51	27	16	7
		65-79	284	35	29	27	9
		80+	196	24	29	39	7
Colon/rectum	Men	50-64	1254	50	26	14	11
		65-79	2029	28	33	31	7
		80+	522	22	33	37	8
	Women	50-64	916	53	27	10	10
		65-79	1733	35	32	24	9
		80+	773	25	30	35	10
Pancreas	Men	50-64	156	35	32	18	15
		65-79	274	27	30	35	8
		80+	45	27	31	33	9
	Women	50-64	105	47	28	12	13
		65-79	255	29	34	27	9
		80+	85	24	31	34	12

Table 3 continued

Lung	Men	50-64	1945	39	32	21	8
		65-79	3478	22	35	38	5
		80+	518	21	31	40	8
	Women	50-64	673	45	32	15	8
		65-79	741	27	33	33	8
		80+	94	32	27	33	9
Breast	Women	50-64	3247	60	21	7	12
		65-79	2655	37	31	22	10
		80+	824	25	33	33	9
Cervix uteri	Women	50-64	125	57	26	9	8
		65-79	87	44	32	18	6
		80+	37	27	30	32	11
Corpus uteri	Women	50-64	507	54	27	10	9
		65-79	490	31	34	28	7
		80+	126	19	34	42	5
Ovary	Women	50-64	380	57	26	8	9
		65-79	386	36	33	24	7
		80+	102	29	36	25	9
Prostate	Men	50-64	1158	45	27	11	18
		65-79	3316	30	33	22	15
		80+	784	27	32	25	16
Bladder	Men	50-64	350	39	30	16	16
		65-79	804	22	38	31	9
		80+	248	18	35	38	9
	Women	50-64	87	30	38	14	18
		65-79	186	32	30	31	8
		80+	109	22	31	29	17
Kidney	Men	50-64	263	42	32	16	10
		65-79	315	22	34	34	10
		80+	50	8	34	44	14
	Women	50-64	157	42	31	13	13
		65-79	241	23	35	32	10
		80+	58	17	31	43	9
NHL	Men	50-64	268	50	24	15	11
		65-79	335	29	36	31	5
		80+	63	14	41	33	11
	Women	50-64	201	54	24	10	12
		65-79	280	36	33	26	6
		80+	98	32	30	36	3

Table 4. Age-specific prevalence (%) of the most common serious concomitant diseases among newly diagnosed patients with 13 major cancers, 1995-2001. Source: Eindhoven Cancer Registry.

		other cancers			heart /vascular disease			COPD			hypertension			diabetes		
age (yrs)		50-64	65-79	80+	50-64	65-79	80+	50-64	65-79	80+	50-64	65-79	80+	50-64	65-79	80+
tumor	Sex	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Esophagus	M	7	19	26	15	35	32	12	16	23	15	20	9	5	11	16
	W	11	16	16	4	16	16	14	9	8	10	17	24	4	13	14
Stomach	M	6	12	20	19	33	26	7	14	16	15	15	19	6	9	12
	W	10	13	15	8	15	25	4	9	7	14	26	29	7	14	20
Colon/rectum	M	7	15	22	13	28	31	6	15	16	16	20	15	7	10	12
	W	10	14	16	5	14	24	5	8	9	15	25	25	6	14	16
Pancreas	M	6	13	13	22	26	33	5	17	16	12	18	18	13	20	22
	W	8	9	7	8	15	27	3	9	6	15	27	28	11	27	25
Lung	M	9	16	17	18	33	31	20	29	31	10	15	10	6	10	11
	W	9	15	14	11	21	21	21	24	14	12	22	18	5	12	10
Breast	W	6	10	14	4	12	21	4	6	7	13	29	26	5	13	16
Cervix uteri	W	5	8	11	6	16	22	5	6	3	13	21	27	9	15	32
Corpus uteri	W	8	10	19	3	12	23	4	6	12	23	38	33	8	22	25
Ovary	W	12	13	15	4	15	16	5	7	5	13	26	26	4	12	12
Prostate	M	6	9	14	12	23	27	6	12	14	13	17	11	5	8	9
Bladder	M	19	24	30	16	28	28	8	15	21	9	16	13	4	11	11
	W	24	23	17	8	13	24	9	7	8	11	31	18	7	17	15
Kidney	M	12	16	28	15	32	34	6	15	16	16	25	18	8	10	20
	W	7	18	17	6	18	28	8	6	3	27	32	45	9	18	22
NHL	M	5	13	17	14	28	35	4	15	10	12	18	11	5	10	11
	W	6	15	15	6	11	24	2	8	4	15	25	21	7	11	13

Table 5. Influence of age and co-morbidity on primary treatment, according to tumor type and/or stage

Tumor	Stage	Influence of rising age (> 60 yrs)	Influence of co-morbidity
Colon	Dukes A/B	None	None
	Dukes C/D	Less adjuvant CT, none >80yrs	Age 65-79: less adjuvant CT
Rectum	Dukes B/C	Less adjuvant RT	Less adjuvant RT
NSCLC*	I/II	Less surgery, more RT alone	Age 60-79: less surgery
	III/IV	Less CT	None
SCLC**	Limited	Less CT+RT, more CT alone	Age 70-79: less CT+RT
	Extensive	Less CT, more abstinence	None
Breast		Less surgery, more endocrine	Less adjuvant RT, more endocrine
Prostate		Less prostatectomy, more endocrine	Age 60-79: less prostatectomy, more endocrine
NHL***	Indolent	Less CT, more RT and wait & see	None
	Aggressive	Less CT, more abstinence	Age 70+: less CT

* Non-small cell lung cancer

** Small cell lung cancer

*** Non-Hodgkin's lymphoma

CT=chemotherapy

RT=radiotherapy

Source: Eindhoven Cancer Registry

The proportion of patients with localized non-small cell lung cancer who underwent surgery with or without radiotherapy was only 9% of those aged 80 or older versus 92%, 79% and 61% of the age groups <60, 60-69 and 70-79, respectively. Patients aged 60-69 and 70-79 received less surgery in the presence of co-morbidity. Most patients with non-localized non-small cell lung cancer received only radiotherapy. The proportion receiving chemotherapy (with or without radiotherapy) was considerably higher among patients younger than 60 (24%) than among those aged 80 or older (2%). Older patients more often did not receive oncological treatment. The number of co-morbid conditions had no substantial influence on treatment chosen for patients with non-localized disease. Elderly patients with limited small cell lung cancer received less adjuvant radiotherapy and more chemotherapy alone. Among patients aged 70-79 with limited small cell lung cancer the proportion receiving adjuvant radiotherapy also decreased in the presence of co-morbidity.

Among patients with breast cancer younger than 80 years over 90% underwent surgery, compared with only 74% of those aged 80 or older. The proportion receiving systemic treatment (mostly chemotherapy for those below 50 years and endocrine treatment for those aged 50 or older) increased from about 40% of those younger than 80 to 57% of patients aged 80 or older. In the presence of co-morbidity less patients received adjuvant radiotherapy (50% of patients with co-morbidity compared to 65% of those without co-morbidity) and more older women received endocrine treatment only.

The number of prostate cancer patients undergoing prostatectomy decreased with increasing age, from 42% of patients younger than 60 to 1% of patients aged 80 or older. The proportion of patients receiving curative radiotherapy also decreased from 17% among those at middle age to 4% of those aged 80 or older. With the rise of age prostate cancer patients received more often hormonal therapy: from 19% of patients below 60 to 59% of patients aged 80 or older. Among patients aged 60-69, the proportion who underwent prostatectomy decreased significantly with co-morbidity from 31% of patients without co-morbidity to 18% of patients with two or more comorbid conditions. In those aged 70-79, these percentages were 8% and 3% respectively. The proportion of patients aged 60-69 receiving hormonal therapy increased from 22% of patients without co-morbidity to 27% of those with two or more co-morbid conditions. In those aged 70-79, these proportions were 36% and 41%, respectively. In the other age groups (< 60 years and 80+ years) there was no significant influence of co-morbidity on treatment choice.

Among patients with non-Hodgkin's lymphoma the proportion receiving chemotherapy decreased with age. For patients with indolent disease the proportion receiving chemotherapy decreased from 60% of patients younger

than 70 to 40% of those aged 70 or older. For patients with aggressive disease the proportion receiving chemotherapy decreased from about 80% to about 60%. Among patients with aggressive disease aged 70 or older the proportion receiving chemotherapy also decreased with co-morbidity.

2.3 Prognosis (see Table 6)

Five-year relative survival rates for colon cancer patients aged 70 or older without co-morbidity exceeded those of patients younger than 70: 75% versus 61%. Relative survival decreased in the presence of co-morbidity, especially for patients aged 70 or older and in case of COPD. Rectal cancer patients younger than 70 exhibited a 5-year survival rate of 65%, which amounted to 62% for patients aged 70 or older. For the latter, the presence of diabetes and cardiovascular diseases lowered 5-survival to 34%. One-year relative survival rates of patients with localized non-small cell lung cancer (NSCLC) were clearly lower for older patients: a 1-year survival rate of 81% for patients younger than 70 and 62% for patients aged 70 or older. The presence of COPD and diabetes affected survival negatively. Survival of non-localised NSCLC was mostly affected by the presence of diabetes. Although survival of small cell lung cancer was strongly related to age at diagnosis, co-morbidity did not seem to have a clear prognostic impact. Breast cancer patients without co-morbidity exhibited 5-year relative survival rates of 86% (when younger than 70) and even 90% (aged 70 or older). But the presence of diabetes and cardiovascular diseases lowered 5-year survival rates of patients aged 70 or older substantially: 56% and 58%, respectively. Prostate cancer patients without co-morbidity had a 5-year survival rate of 88% (no difference between younger and older patients), whereas diabetes and COPD had a negative impact on survival of patients aged 70 or older. Indolent non-Hodgkin's lymphoma patients younger than 70 without co-morbidity had a 1-year survival rate of 94%, versus 80% of patients aged 70 or older. One-year survival of aggressive non-Hodgkin's lymphoma without concomitant diseases at diagnosis was 80% for patients younger than 70 and 73% for patients aged 70 or older. The presence of cardiovascular diseases lowered 1-year survival to 51% for patients younger than 70 and to 43% for patients aged 70 or older.

		Relative survival (SE)			
		<70 years		>70 years	
Tumor type	**Co-morbidity**	1-year	5-year	1-year	5-year
Colon	**None**	**0.82 (1)**	**0.59 (2)**	**0.82 (2)**	**0.75 (4)**
	Diabetes	**0.77 (4)**	**0.51 (6)**	**0.67 (3)**	**0.50 (5)**
	COPD	**0.73 (4)**	**0.47 (6)**	**0.68 (4)**	**0.41 (6)**
	Cardiovascula	**0.74 (4)**	**0.54 (5)**	**0.68 (3)**	**0.45 (4)**
Rectum	**None**	**0.89 (1)**	**0.65 (2)**	**0.80 (3)**	**0.62 (5)**
	Diabetes	**0.78 (5)**	**0.46 (8)**	**0.73 (5)**	**0.34 (7)**
	COPD	**0.87 (4)**	**0.53 (8)**	**0.68 (5)**	**0.40 (7)**
	Cardiovascula	**0.80 (4)**	**0.55 (6)**	**0.72 (4)**	**0.34 (6)**
NSCLC* localised	**None**	**0.81 (3)**	***0.68 (3)***	**0.62 (6)**	***0.46 (6)***
	Diabetes	**0.75 (6)**	***0.59 (7)***	**0.57 (7)**	***0.29 (7)***
	COPD	**0.77 (3)**	***0.60 (4)***	**0.53 (4)**	***0.29 (4)***
	Cardiovascula	**0.76 (4)**	***0.65 (4)***	**0.59 (5)**	***0.39 (5)***
NSCLC* non-local	**None**	**0.29 (2)**	***0.13 (1)***	**0.21 (3)**	***0.10 (2)***
	Diabetes	**0.29 (5)**	***0.16 (4)***	**0.11 (4)**	***0.03 (2)***
	COPD	**0.26 (3)**	***0.10 (2)***	**0.22 (3)**	***0.09 (2)***
	Cardiovascula	**0.26 (3)**	***0.11 (2)***	**0.21 (3)**	***0.07 (2)***
SCLC**	**None**	**0.41 (3)**	***0.10 (2)***	**0.15 (4)**	***0.05 (2)***
	Diabetes	**0.28 (6)**	***0.06 (3)***	**0.15 (7)**	***0.04 (4)***
	COPD	**0.33 (4)**	***0.17 (4)***	**0.19 (4)**	***0.06 (2)***
	Cardiovascula	**0.31 (4)**	***0.10 (3)***	**0.18 (4)**	***0.09 (3)***
Breast	**None**	**0.99 (0)**	**0.86 (1)**	**0.98 (0)**	**00.. (3)**
	Diabetes	**0.95 (2)**	**0.82 (4)**	**0.88 (2)**	**0.56 (5)**
	COPD	**0.97 (2)**	**0.82 (4**	**0.92 (3)**	**0.71 (8)**
	Cardiovascula	**0.93 (2)**	**0.76 (4)**	**0.91 (2)**	**0.58 (5)**
Prostate	**None**	**0.99 (1)**	**0.88 (2)**	**0.99 (1)**	**0.89 (4)**
	Diabetes	**0.92 (3)**	**0.74 (7)**	**0.93 (3)**	**0.64 (7)**
	COPD	**0.92 (30**	**0.81 (5)**	**0.86 (3)**	**0.63 (5)**
	Cardiovascula	**0.95 (2)**	**0.78 (4)**	**0.90 (2)**	**0.70 (4)**
NHL*** indolent	**None**	**0.94 (2)**	***0.90 (3)***	**0.80 (9)**	***0.78 (10)***
	Diabetes	**0.88 (11)**	***0.77 (16)***	**0.73 (17)**	***0.79 (19)***
	COPD	**0.92 (9)**	***0.84 (12)***	**0.86 (19)**	***0.46 (25)***
	Cardiovascula	**0.95 (6)**	***0.71 (12)***	**0.79 (12)**	***0.78 (14)***
NHL*** aggressive	**None**	**0.80 (3)**	***0.68 (3)***	**0.73 (7)**	***0.61 (8)***
	Diabetes	**0.64 (11)**	***0.65 (11)***	**0.70 (11)**	***0.58 (13)***
	COPD	**0.80 (11)**	***0.81 (11)***	**0.56 (12)**	***0.38 (12)***
	Cardiovascula	**0.51 (9)**	***0.41 (9)***	**0.43 (8)**	***0.42 (8)***

Table 6. Relative 1- and 5-year survival rates, according to age and co-morbidity. Source: Eindhoven Cancer Registry. SE= standard error. * Non-small cell lung cancer. ** Small cell lung cancer. *** Non-Hodgkin's lymphoma. *# 2-year survival*

3. DISCUSSION

3.1 Validity of Data

Co-morbidity is a multidimensional variable with a variation in severity. Diseases that influence mortality may not be the same as those influencing function or tolerance to treatment. Although there is general agreement about the importance of co-morbidity for cancer management and prognosis, there is no consensus about the types of diseases that should be included, nor about the weighing of the conditions. There are several methods for determining the total score of diseases. The most global measure is the sum of the number of conditions present. Secondly, a severity score can be assigned to each condition and the total score is the summation of all the severity scores present in a patient at a certain moment. A third method is to assign a severity score to each condition and the total severity is based on the most severe condition present in a patient. When a patient has more than one disease, there may also be a multiplicative or synergistic effect on outcomes, and grading severity according to only the sum of diseases or scores or to only the single most severe condition may miss the burden that multiple chronic diseases can place on an individual. Several systems have been proposed, each with its own classification and scoring system. The choice of the classification system is dependent on the aim of the study, the clinical problem to be explored. The five most widely used systems are: the indexes of Kaplan-Feinstein [9], Charlson [6], and of the National Institute of Ageing/National Cancer Institute (NIA/NCI) [10], the Cumulative Illness Rating Scale-Geriatric (CIRS-G) [11], and the Index of Co-Existent Diseases (ICED) [12]. In the Eindhoven Cancer Registry an adapted version of the Charlson co-morbidity index was chosen for the following reasons: it was the most widely used validated classification system at the time and it is relatively easy to use [4, 13]. We wanted to avoid the plethora of minor conditions, each with their classification problems and changes in the natural history. Scoring needed to be done by cancer registry personnel trained in oncological diagnoses who could only spend a limited amount of time on this.

For the assessment of co-morbidity several sources can be used. Although the medical record is generally regarded as the most complete source of information on the patient's past and current health status, there may be some limitations in using medical records, such as differences in information in the records between hospitals or specialists, or possible selection bias due to differences in the number of physician visits. Data on co-morbidity can also be gathered from administrative medical record databases or discharge data. In a comparison between the Charlson co-morbidity index derived from medical records with that derived from databases of administrative medical

records, the data derived from the medical records had a better predictive value than the administrative disease data[7].

Between 2001 and 2003 the completeness and accuracy of our data on co-morbidity in the Eindhoven Cancer registry were validated in a random sample of 2607 patients with colorectal, lung, breast and prostate cancer and non-Hodgkin's lymphoma aged 40 and older and diagnosed between 1995 and 1999. Co-morbidity scored by the registry team was compared with that scored by a team of a surgeon and an epidemiologist. Recording of co-morbidity proved to be entirely correct for almost 70% (ranging from 59% to 72%) of patients. Some under-registration occurred especially of cardiovascular conditions (Internal report, 2002). This appeared to be mainly due to the use of unknown terminology, unknown abbreviations or illegible handwriting of the specialist. Although the unregistered conditions were at the time not very severe, this would imply that the real effects of co-morbidity on treatment and survival are probably stronger than those presented in this chapter and in our publications.

3.2 Prevalence

The higher prevalence of co-morbidity among older patients was expected, because the prevalence of chronic diseases generally increases with age. The prevalence of co-morbidity among older patients may even be underestimated due to ascertainment bias. Younger patients underwent surgery more often and received chemotherapy more often. The prevalence of co-morbidity reported by the treating physician might then be more elevated among younger patients, due to the required screening examinations before treatment.

The high risk of cardiovascular diseases and chronic obstructive pulmonary diseases for patients with cancer of the esophagus, stomach, lung, bladder and kidney can be explained by the high proportion of smokers among these patients, especially men [14, 15]. That diabetes mellitus occurred in a high proportion of patients with cancer of the pancreas is not surprising [16, 17]. A history of diabetes has been consistently associated with a two-fold increased risk for endometrial cancer [18], probably because both are related with obesity. The increased prevalence of diabetes among patients with cervical cancer was more strongly related to squamous cell carcinoma than to adenocarcinoma. Kidney cancer has been associated with hypertension, although it is unknown whether this results from the hypertension itself or from the anti-hypertensives [19]. Previously, we also found an association of hypertension with the incidence of gliomas [20]. The risk of renal cancer is also elevated among patients with diabetes[21].

The prevalence of cardiovascular diseases and pulmonary diseases was higher among men compared to women, which can be largely explained by a

higher prevalence of smoking among men in the past. By contrast, the prevalence of hypertension (a less serious condition) and diabetes was higher among women.

3.3 Treatment

If alternative treatment strategies were available, older patients were often treated less aggressively than younger patients. After stratification for age, the influence of age and co-morbidity on treatment choice differed, according to tumor type.

When surgery is inevitable like in patients with colorectal cancer, higher age or the prevalence of co-morbidity did not have any influence on the resection rate. On the other hand, older patients with non-small cell lung cancer (with serious co-morbidity) more often received radiotherapy instead of surgery [22]. Surgical mortality increases markedly with age and is especially high for pneumonectomy [23, 24]. The resection rate also declined with co-morbidity, probably because of the expected higher incidence of postoperative complications and mortality [25]. However, in everyday practice the resectability is not determined primarily by co-morbid conditions, but by its effects on lung function and cardiac function. The resection rate for prostate cancer also decreased with co-morbidity, whereas the proportion receiving hormonal treatment increased[26].

Administration of adjuvant chemotherapy markedly decreased with rising age and co-morbidity for patients with Dukes C or D colon cancer [27], probably because of the higher rate of hematological complications [28,29,30]. Administration of primary chemotherapy also decreased with age and co-morbidity in patients with non-Hodgkin's lymphoma[31].

Administration of adjuvant radiotherapy decreased with age and co-morbidity in patients with rectal cancer, limited small cell lung cancer or breast cancer [13, 27, 32]. However, we did not find that the rate of expected complications of radiotherapy was higher for older patients with co-morbidity. Therefore, the reluctance of offering adjuvant radiotherapy might be related to practical reasons like the distance to a radiotherapy institute or the burden of the 20 to 30 visits to the radiotherapy institute.

Several authors also found less aggressive treatment of patients with co-morbidity in case of breast cancer, colorectal cancer, or prostate cancer[33,34, 26, 35, 36].

Age seemed to have more influence on treatment chosen than co-morbidity. Apparently, co-morbidity alone does not entirely explain why elderly non-small cell lung cancer patients and prostate cancer patients underwent surgery less often and why those with colon cancer, rectal cancer, small cell lung cancer and breast cancer received less (adjuvant) chemotherapy or adjuvant radiotherapy. Performance status, the psychological condition of

the patient, social factors and patient's decision, families decision or doctor's decision may also play a role [33, 2]. The lower proportion of elderly patients undergoing surgery or receiving chemotherapy also appeared in another area of the Netherlands[37].

3.4 Survival

For most tumor types relative survival for those without co-morbidity did not decrease with age, except for patients with lung cancer or non-Hodgkin's lymphoma. The outcome of patients without co-morbidity could be comparable to the outcome of patients in clinical trials, because those with co-morbidity are often excluded from clinical trials.

For patients with lung cancer co-morbidity had no independent prognostic effect [22]. This contradicts some other studies [38-41], but they were not population-based. They also used other scales for measuring co-morbidity: the Kaplan-Feinstein Index [9] and the Cumulative Illness Rating Scale-Geriatric (CIRS-G) [11]. In one of the studies, co-morbidity affected overall survival in surgically resected stage I NSCLC patients, when co-morbidity was rated according to CIRS-G, but not according to the Charlson scale [39]. In another American study co-morbidity count and the Charlson index were significant predictors for lung cancer survival, but only explained 2.5% and 2.0% of the survival variation, respectively [42]. Probably the influence of co-morbidity on survival is of less importance in the case of a lethal disease such as lung cancer. Most of these patients die from lung cancer, before they become at risk of dying from the co-morbid condition.

For the other tumors, co-morbidity had an independent prognostic effect. This negative influence of co-morbidity on survival of cancer patients might be due to several mechanisms: the increased risk of death due to the co-morbid condition itself, more contra-indications for anti-cancer treatment, more indications for dose reduction and a higher rate of treatment-related complications such as infections and cardiovascular events. In several of our recent studies the adverse effects of co-morbidity on survival appeared to be independent of treatment, so less aggressive treatment could not (fully) account for the observed differences in survival between patients with and without co-morbidity [22, 27, 32, 31]. The minor effects of cardiovascular conditions on relative survival of lung or colon cancer may be explained by earlier detection of the cancer through surveillance (X-thorax) or early bleeding of polyps by thrombolytic therapy. Some studies have shown that performance status and co-morbidity are both independent prognostic factors [1, 2], which therefore may need to be included in future prognostic studies, supplemented by the psychological or mental condition of the patient, and the patient's and/or family decision or even doctor's decision should be included.

4. CONCLUSIONS

There is now clear evidence that the prevalence of co-morbidity among older cancer patients is high and that older patients (with co-morbidity) are often treated less aggressively, which seems to have a negative influence on survival. However, would outcomes really improve if more patients were treated, according to the guidelines that were developed on the basis of results in groups of younger patients without co-morbidity? Would more complications occur in older patients with co-morbidity? If that is the case, is it possible to develop special treatment regimens for older cancer patients with co-morbidity and adapt the guidelines? It remains relevant to study the influence of age and co-morbidity on toxicity from treatment, quality of life and prognosis in unselected groups of patients.

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Chapter 6

HEMOPOIESIS AND AGING

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Hemopoiesis is central to the study of cancer and aging for the following reasons: (1) to some extent, hemopoiesis is a sensor of physiologic aging. In particular, hemopoiesis may be inhibited by the same catabolic cytokines that accumulate in the circulation with aging and predict functional decline and death, [1-4] (2) hemopoiesis is one of the main targets of cytotoxic chemotherapy [1,5]. A decline in hemopoietic reserve may compromise the ability to administer chemotherapy in adequate doses to older individuals [6]. In addition, anemia is an independent risk factor for chemotherapy-induced myelosuppression,[7-10] and, (3) anemia has a number of detrimental effects on the older person that include increased risk of mortality [11-14] and disabilities [15-18] . Thus, anemia may lessen the benefits and enhance the risk of cancer treatment.

In this chapter we examine the influence of aging on hemopoiesis, the risk of myelosuppression following cytotoxic chemotherapy in the older aged person, the prevalence, incidence, mechanisms and consequences of anemia in the older person, and the management of anemia and of hemopoietic complications of cancer treatment.

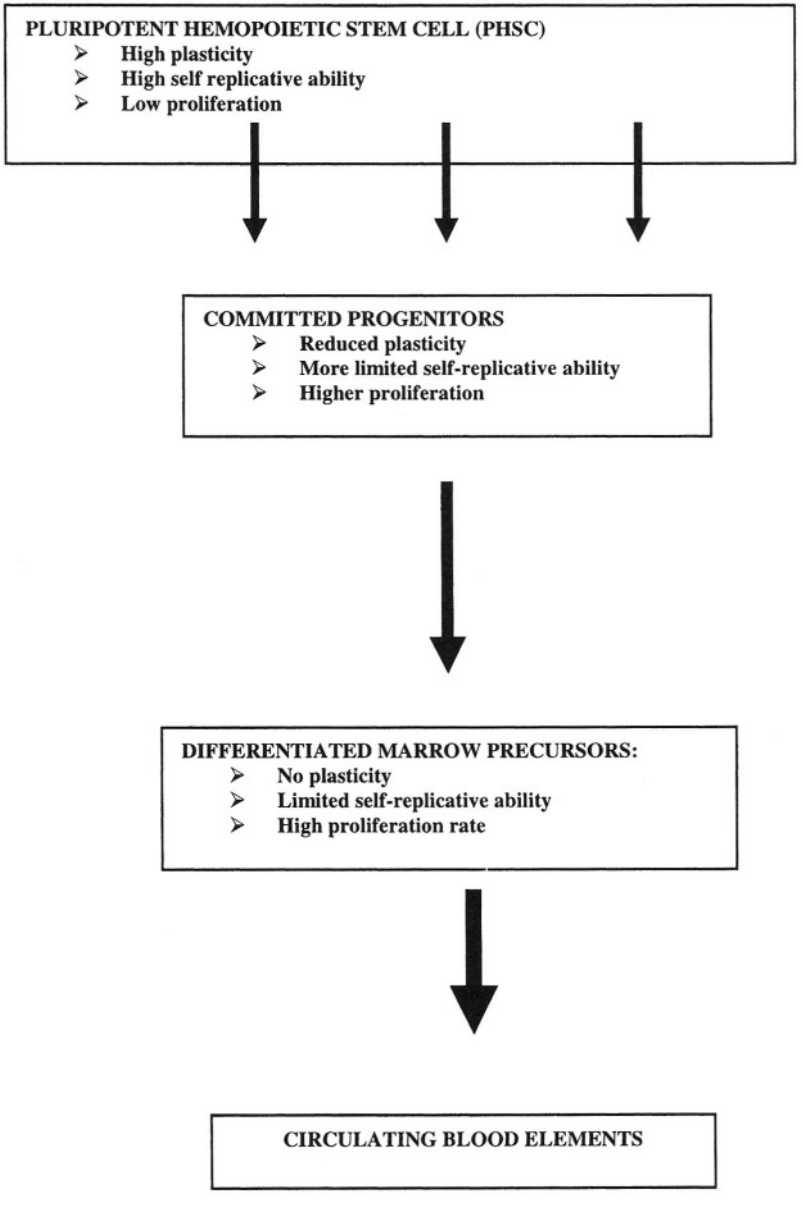
1. AGE AND HEMOPOIESIS

Hemopoiesis is compartmentalized (Figure 1)[1] The homeostasis of the peripheral blood, that is a balance between losses and production of circulating blood elements involves the integrity and the coordination of a number of serial processes. The pluripotent hemopoietic stem cells (PHSC) need commit into hemopoietic progenitors and at the same time maintain their own population by self-replication. The high plasticity of the PHSC that may give origin to different lines of blood elements while replicating themselves is largely lost by the committed progenitors which do differentiate only into one hemopoietic line, giving origin to the recognizable marrow precursors that mature into circulating blood elements[1]. Commitment, differentiation, and maturation are modulated by hemopoietic cytokines and made possible by the hemopoietic stroma. The roles of the stroma include homing of PHSC and committed progenitors, and production of some of the cytokines that modulate growth and differentiation. Hemopoietic insufficiency may be caused by one or more of these mechanisms: exhaustion of PHSC, inadequate production of growth factors or excess production of inhibitory substances, decreased sensitivity of PHSC and hemopoietic progenitors to the growth factors and disruption of the hemopoietic microenvironment.

1.1 Aging and PHSC Reserve

The influence of aging on PHSC reserve is controversial. Both experimental and clinical observations suggest the self-renewal of PHSC is exhaustable. In the murine model the ability of forming hemopoietic colonies in the spleen declined with serial stem cell transplants [19]. Telomere length and telomerase activity of PHSC underwent a progressive decline with age, [20] and hemopoietic stress was associated with a reduction in the concentration of PHSC of older, but not of younger animals [21-22]. Hemopoietic stress consisted of isolation [21] or of sepsis [22]. In humans, the hemopoietic tissue becomes progressively reduced with age, [23] while mortality from infection increases, which suggests decline in neutrophil reserve [24]. The number of CD 34+ cells, that may reflect the reserve of pluripotent hemopoietic progenitors [25] and the self-replicative ability of these elements appear to decline with age [26]. Hemopoietic stress in human reveals some degree of hemopoietic insufficiency: the injection of granulocyte-macrophage colony stimulating factors (GM-CSF) in healthy individuals aged 70 and older and 30 and younger, produced a much lower increment in

Figure 1. Overview of hemopoiesis as a compartmental process.



the concentration of pluripotent hemopoietic progenitors in the circulation of the aged, despite that the baseline concentrations of these elements were the same in both groups, [27] and the risk of neutropenia, neutropenic infections and thrombocytopenia following cytotoxic chemotherapy increased after age 60 [28-39]. The fact that the prevalence and incidence of anemia of unknown causes increase with age may also be construed as evidence of progressive hemopoietic insufficiency [40-43].

These observations support an age-related decline in hemopoietic reserve that may be of consequence in the presence of severe and prolonged hemopoietic stress. In condition of homeostasis, the hemopoietic activity appears adequate to preserve a normal peripheral blood count throughout a person's lifetime, in the majority of individuals [44-45]. Even among the oldest old, it should not be assumed that anemia, neutropenia, thrombocytopenia or pancytopenias are a natural consequence of age.

1.2 Age and Production of Hemopoietic Cytokines

The relationship between age and production of hemopoietic cytokines is not clear. In the murine model, utilizing transgenic mice undergoing early senescence (senescence-accelerated mice or SAM), some authors reported increased production of colony inhibiting activity (CIA) in response to lypopolysaccaride [46]. Of interest, lypopolysaccaride induced production of colony stimulating activity, followed by CIA in young animals. This observation suggests that age may lead to a loss in ability to produce hemopoietic growth factors, while the ability to produce CIA is unaffected and maybe enhanced.

In humans the data are inconclusive. Whereas the production of GM-CSF from monocytes grown "in vitro" seem to decline with the age of the cell, [47] recent studies demonstrated a decline in GM-CSF production following exposure to phytohemagglutinines only by monocytes obtained from healthy centenarians [25]. An age effect was not observed in younger subjects up to age 73.

Several studies explored the production of erythropoietin in the aged, with conflicting results. In some cases of anemia of unknown causes in older individuals, the circulating levels of erythropoietin were inadequate for the degree of anemia; [40, 48-52] but the Glomerular Filtration Rate (GFR) of these patients had not been measured. It is reasonable to expect that [54-61] reduction in GFR, common with aging [53] might have accounted for the poor erythropoietin response to anemia. In other studies comparing the concentration of circulating erythropoietin in younger and older individuals

with sideropenic anemia the level of erythropoietin were normal and even increased among the old ones [54, 55]. In a rare form of age-related anemia (dysautonomic anemia)[52] the production of erythropoietin is decreased.

The production of hemopoietic cytokines in the aged should be seen in the context of the increased concentration of catabolic cytokines in the circulation of some elderly individuals. Recent studies demonstrated that increased concentrations of Interleukin 6 are associated with functional decline, increased risk of geriatric syndromes and of death [2, 54-61]. Increased levels of IL-6 are also present in patients with anemia and IL6 appears to inhibit both the response of erythropoietic progenitors to erythtopoietin and the production of erythropoietin [3, 62]. IL-6 and possibly other catabolic cytokines such as tumor necrosis factor (TNF), interleukin 1 (IL1) and Interleukin 10 (IL10) may be responsible of a relative hemopoietic insufficiency by reducing both the production of growth factors and the sensitivity of hemopoietic progenitors to these factors [3, 22, 46, 63, 64]. The catabolic effects of these cytokines may be enhanced by reduced secretion of Growth Hormone[63, 64].

1.3 Aging and Sensitivity to Hemopoietic Cytokines

The information related to this issue is very limited and circumstantial. The age-related progressive loss in ability to respond to hemopoietic stress might be explained in part by a loss of sensitivity to hemopoietic cytokines. It is also well known that catabolic cytokines and in particular IL-6 and TNF blunt the response of erythropoietic progenitors to erythropoietin [3, 61]. Other observations supporting a reduced sensitivity to hemopoietic growth factors include the fact that erythropoietic enhancement observed "in vitro" with addition of indocin to the culture appears diminished in the marrow from older individuals,[65, 66] that the generation of the same reticulocyte response requires higher levels of erythropoietin in anemic older than in younger patients [67-68]. Contrasting with these findings, Bagnara et al [25] found that *in vitro* responsiveness of pluripotent hemopoietic precursors to erythopoietin, G-CSF and GM-CSF was well maintained even in persons aged 100 and older. While there are circumstances that may reduce the sensitivity of erythopoietic progenitors to hemopoietic cytokines, this does not appear a generalized occurrence with aging. From a clinical standpoint it is important to realize that a good response to pharmacological doses of G-CSF, GM-CSF (69), Interleukin 11, [70] megakaryocite growth stimulating factors (M-CSF)[71] and erythropoietin[72] may be seen irrespective of the age of the patient.

1.4 Aging and Hemopoietic Microenvironment

The consequences of aging on the function of the hemopoietic microenvironment are largely unknown. As autologous stem cell rescue after high dose chemotherapy has been carried on successfully in persons aged 70

and older with multiple myeloma, [73, 74] it is reasonable to assume that the ability to home stem cells persists to some extent beyond age 70.

2. AGE AND HEMOPOIETIC COMPLICATIONS OF CYTOTOXIC CHEMOTHERAPY

At this point it is legitimate to ask whether myelosuppression following cytotoxic chemotherapy becomes more prolonged and severe with age, as a consequence of progressive reduction in hemopoietic reserve and whether the risk of increased toxicity may be ameliorated, in order of preserving the full dose and the full benefits of chemotherapy.

The risk and severity of myelotoxicity was not increased in patients aged 65-70 and older in at least six large clinical trials (Table 1)[75-80]. While these studies demonstrate that the risk of myelosuppression is not increased in all older individuals, they shed little light on the risks of chemotherapy in the general aged population. The percentage of patients aged 80 and older was negligible, and also patients aged 70 and
older were underrepresented: whereas 40% of cancers occur in these age group, older individuals made up only 10-15% of patients enrolled in these clinical trials. Clearly, the older patients were highly selected as it is to be expected in studies conducted in major cancer centers or by cooperative oncology groups. Furthermore, the retrospective nature of these studies may have prevented the detection of small age-related differences.

Several other studies support increased risk of myelodepression among the elderly [29-32]. The risk of neutropenia was increased for women 65 and older receiving adjuvant chemotherapy for breast cancer with doxorubicin and cylophosphamide [32]. Of special interest, myelodepression was cumulative in the older but not the younger patients, suggesting impairment of hemopoietic reserve with age [32]. A review of the experience of the South West Oncology Group (SWOG) [29] and of the International Breast Cancer Study Group [30] showed that age 65 and older was a risk factor both for myelotoxicity and lower chemotherapy dose intensity. The once popular regimen MACOP-B (methotrexate, adriamycin, cyclophosphamide, oncovin prednisone and bleomycin) for Large cell Non-Hodgkin's Lymphoma has produced increased incidence of neutropenia, neutropenic infections, and infectious mortality in patients aged 60 and older[33]. Armitage et al reported a

30% mortality rate among individuals aged 70 and over treated with CHOP for non-Hodgkin's lymphoma [28].

A number of prospective trials exploring different forms of chemotherapy in elderly Non-Hodgkin's lymphoma patients (Table 2) [28, 31, 34-39, 81-82] reported a risk of severe neutropenia around 50% and a risk of

Table 1. Age and myelotoxicity of cancer chemotherapy: results of five retrospective trials.

Author	Patients #	Patients ≥ 70 (%)	Source
Begg & Carbone, 1983 (75)	5459	780 (13%)	ECOG data base
Gelman & Taylor, 1984 (76)	231	31 (13%)	Dana Farber Cancer center: Patients over 65 had been treated prospectively with dose-adjustment for cyclophosphamide and methotrexate and 2/3 FU dose And results compared with 161 fully evaluable younger patients. Patients over 80 experienced shortened survival
Christman et al, 1992 (77)	170	70 (42%)	Piedmont Oncology Group database; high degree of patients selection
Giovannazzi-Bannon et al, 1994 (78)	672	65: 271 (40.3%) 70: ? (25%)	Illinois cancer center phase II trials.
Ibrahim et al, 1996 (79)	1011	65: 244 (24%) 70: ? (,20%)	MD Anderson Hospital patients with metastatic breast cancer aged 50 and older
Ibrahim et al, 2000 (80)	390	65: 65 (18%) 70: ? (< 10%)	M D Anderson hospital patients with breast cancer receiving anthracycline-containing adjuvant chemotherapy

neutropenic infection around 25% among patients aged 60 and older treated with CHOP or CHOP-like regimens. The only exception to theses finding was the study of Doorduijn et al [83] in which the incidence of neutropenic infection was 10%. The difference may probably be accounted in terms of patient selections. In the majority of studies the risk of death varied between 5-15%. Age 60 and over is a risk factor for more prolonged neutropenia, neutropenic infections and infectious death also in the management of Acute Myelogenous Leukemia (AML) [84]. In this case the disease itself may compromise the patient's hemopoietic reserve, because in the elderly with AML the PHSC is involved by the disease in approximately 60% of the times[84, 85].

The risk of chemotherapy-induced thrombocytopenia and anemia in older individuals is less well known. It is important to remember however that anemia may be very deleterious in elderly cancer patients, because anemia by itself is a risk factor for myelosuppression [7-10]. This phenomenon may be accounted for by the pharmacokinetics of different cancer agents that are heavily bound to red blood cells [7, 10]. In presence of anemia the concentration of free drug in the circulation and the risk of toxicity may be

increased. Furthermore, anemia is associated with fatigue that in older individuals is a risk factor for functional dependence [15-18, 86].

Several strategies have been proposed to reduce the risk of chemotherapy-induced neutropenia in the aged. These include dose-reduction, prophylactic oral antibiotics, prophylactic use of hemopoietic growth factors, and correction of anemia.

Dose reduction appears ill advised when dealing with curable cancer. In the case of large cell lymphoma, dose reduction has resulted in inferior outcome [36, 38, 40, 87, 88]. A recent study showing that dose-dense CHOP (every two weeks) was superior in terms of response rate and survival to classical CHOP

Table 2. Incidence of life-threatening neutropenia; neutropenic infections and death in older individuals with large cell Non-Hodgkin's Lymphomas treated with CHOP-like regimens.

Author (s)	Patient #	Regimen	Age	Neutropenia	Neutropenic Fever	Treatment-related Deaths	Growth Factor
Zinzani (34)	161	VNCOP-B	60+	44%	32%	1.3%	-
Sonneveld (35)	148	CHOP CNOP	60+ 60+	NR NR	NR NR	14% 13%	- -
Gomez (31)	26	CHOP	60+ 70+	24% 73%	8% 42%	0 20%	GM-CSF GM-CSF
Tirelli (36)	119	VMP CHOP	70 + 70+	50% 48%	21% 21%	7% 5%	- -
Bastion (37)	444	CVP CTVP	70+ 70+	9% 29%	7% 13%	12% 15%	- -
Doorduijn (83)	152	CHOP	65+	21%	10%	5%	-
Osby (39)	455	CHOP CNOP	60+	91%	47%	n.r	-

every three weeks in lymphoma patients aged 60-75, further emphasizes the importance of the dose even in older patients [89]. In adjuvant treatment of breast cancer the importance of the dose was initially reported by Bonadonna et al, who demonstrated that older women had received lower total doses of CMF than younger women and experienced lower benefits [90]. The CALGB

study also showed that the dose of doxorubicin was critical to outcome [91] and recent studies showed that dose intense treatment was superior to standard treatment in terms of survival and recurrence [92]. In AML the importance of the dose of cytarabine in the consolidation phase has been well established [93, 94]. More controversial is the importance of the doses of anthracyclines in induction, but at least in patients with favorable cytogenetics 60mg /m2 of daunorubicin appear superior to 45 mg/m2 [95-98].

The adjustment of the dose of agents excreted from the kidney to the Glomerular Filtration Rate of individuals patients seems to reduce the toxicity without compromising the outcome of treatment [99] and is a reasonable approach for patients aged 65 and older, in whom a compromise of GFR is more likely. Given the variability of drug pharmakcokinetcs, it should also be recommended that the doses of treatment be increased in the absence of toxicity.

Though the prophylactic use of sulfametoxazol/trimetrophrin resulted in reduction in infection from gastrointestinal pathogens in patients with prolonged neutropenia [100], this strategy appears complementary rather than alternative to the use of hemopoietic growth factors. There is no proof that prophylactic antibiotics may also prevent pseudomonas, staphylococcal, fungal and viral infections. Furthermore, this practice may result in the emergence of resistant bacterial strains.

3. AGE AND ANEMIA

The incidence and prevalence of anemia increase with age [14, 40,101]. The consequences of anemia are twofold: anemia may herald a serious underlying disease, and anemia itself may have serious health consequences including, death, functional dependence, dementia, cardiac failure, and increased risk of complications of pharmacotherapy [7-10].

3.1 Definition of Anemia

The definition of anemia by the World Health Organization (WHO) as hemoglobin levels of less than 13 g/dL in men and less than 12 g/dL in women [40], may be outdated in view of new evidence. A Cohort study of home dwelling women aged 65 and older, the Women's Health and Aging Study, showed hemoglobin levels below 13 gm/dl are independent risk factors for mortality [13] and disability [17]. A correlation between disability and hemoglobin levels below 13gm/dl was also shown by a cohort Italian study of healthy older individuals [16], whereas the EPESE study [15], and a cross-sectional study of 3,946 New Englanders (102) showed that declining hemoglobin is associated with declining function in home dwelling persons

aged 70 and older. Prospective studies of cancer patients treated with erythropoietin showed reduction in fatigue and improvement in energy levels when hemoglobin levels rose above 12 g/dL [103-107].

3.2 Epidemiology of Anemia

The prevalence of anemia increases markedly after age [60 14, 40, 101, 102]. Among the residents of Olmsted County, Minnesota, USA, both prevalence and incidence of anemia started rising by age 65 and rose more steeply after age 80 (Figure 2) [14, 108]. Despite these findings, anemia cannot be considered a common consequence of aging, as the average hemoglobin levels of older individuals without serious conditions, remained stable between ages 60 -98, according to longitudinal and cross-sectional studies [101, 102, 109, 112]. Seemingly, the increased prevalence of anemia reflects increased prevalence of comorbidity and of functional decline. Given a more limited hemopoietic reserve, older individuals may become more susceptible to anemia when faced by hemopoietic stress, such as bleeding, myelosuppressive substances, acute or chronic diseases [2, 113].

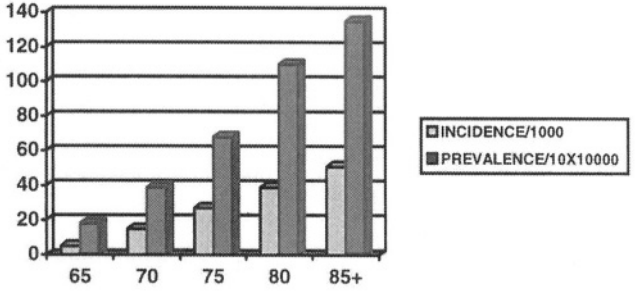


Figure 2. Age-related prevalence and incidence of Anemia in Olmstead County, Minnesota.

One should also remember that qualitative changes of hemopoiesis, such as myelodysplasia, are more common with age [1,3].

The common causes of anemia in the elderly are shown in Table 3, derived from an outpatient [14] and an "in hospital" [42] study. The high prevalence of anemia of unknown causes, reported also by others [41] may reflect inadequate diagnostic work up, early myelodysplasias and absolute or relative erythropoietin deficiency. Absolute erythropoietin deficiency may

result from some degree of renal insufficiency, progressively more common with age [53]. The construct of relative erythropoietin deficiency is illustrated by the comparison of the erythropoietin response to anemia in patients with iron deficiency and in those with chronic disease anemia [48-50,55, 62, 68, 114, 115]. While baseline erythropoietin levels are similar in the two groups of patients for values of hemoglobin higher than 12 gm/dl, for anemic values of hemoglobin, they are lower in anemia of chronic diseases. Impaired erythropoietin secretion and reduced sensitivity to erythropoietin may both contribute to relative erythropoietin deficiency in this condition.

Anemia of chronic disease is probably the most common form of anemia in the elderly [14, 41,42], and is characterized by low serum iron, low or normal TIBC, normal or high serum ferritin levels, and low concentrations of soluble transferrin receptor [115-117].

Table 3. Primary causes of anemia
(From Joosten, E., et al., Gerontology 1992;38:111–117.
with permission from S. Karger AG, Basel).

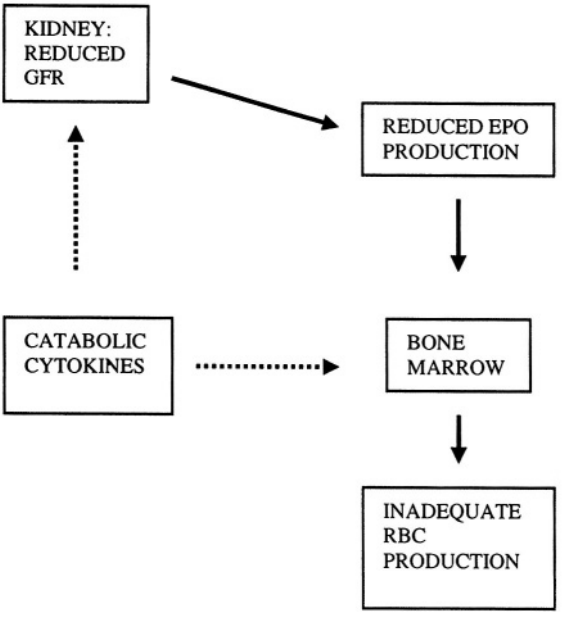
Cause	Prevalence (%) Joosten (Source: hospital discharges)	Anemia Source: Olmsted County Records
Chronic disease	35	17
Unexplained causes	17	36
Iron deficiency	15	15
Post-hemorrhagic	7	7
Renal failure, liver, and endocrine disease	6.5	8
Myelodysplasia or acute leukemia	5.5	-
Chronic leukemia or lymphoma	5.5	-
Vitamin B_{12} or folate deficiency	5.5	-
Other hematological disease	3	17

Patients with this condition cannot mobilize and utilize iron, which is stored in excess in the reticulo-endothelial system. The sensitivity to erythropoietin is reduced in all forms of anemia of chronic diseases, but varies from case to case. Anemia associated with rheumatoid arthritis generally responds to lower doses of erythropoietin than that associated with cancer,[115] suggesting different pathogenesis. In the “cancer type” of anemia

an earlier erythropoietic progenitor may be involved. In addition, red blood cell survival is reduced [115]. Clearly, older individuals appear at increased risk for this form of anemia, due to increased concentration of catabolic cytokines in the circulation, and decline in GFR, that together may conjure relative erythropoietin insufficiency. While this hypothesis has not been completely proved yet, it is clear that the production of erythropoietin in response to anemia is inadequate in some older individuals [46-50].

Iron Deficiency Anemia is due to chronic blood loss whose possibility should always be investigated. Especially among oldest individuals a source of blood loss may not be found and the possibility of inadequate iron absorption should be entertained [41,42,117]

Figure 3. Pathogenesis of relative erythropoietin insufficiency



This appears particularly likely in individuals with lower iron stores, such as women, vegans, and individuals who had experienced multiple bleeding episodes earlier in life.

Iron deficiency is documented by low serum ferritin levels, high total iron binding capacity (TIBC) and transferrin levels, low transferrin saturation, high concentration of free transferrin receptor, and absent bone marrow iron stores [117].

Anemia of Renal Insufficiency is also likely in some older individuals, given a progressive decline in GFR with age [53]. As already discussed in the case of anemia of chronic disease, renal insufficiency may contribute to anemia, rather than being the only cause.

A deficiency in vitamin B_{12} should be suspected in all elderly individuals with circulating levels of cobalamine within the lower limits of normal but equal to or lower than 300 pg/ml [118-120]. In these cases elevated circulating levels of methyl malonic acid or histidine, indicate B_{12} deficiency [118]. The prevalence of B_{12} deficiency may be as high as 15% after age 60 and is rarely due to pernicious anemia: in the majority of cases it results from decreased digestion of food bind vitamin B_{12} due to increased gastric pH and reduced pepsin production [118-120]. In the majority of elderly individuals B12 deficiency may be present in the absence of anemia, if folate levels are normal and is manifested by neurological findings including peripheral neuropathy, posterior column dysfunction and reduced cognition [120].

3.3 Clinical Implications of Anemia

At least five studies have shown that anemia is an independent risk factor of mortality [11-14,121]. Of these the report of Chaves et al is particularly provocative [13] as the risk of mortality started increasing for hemoglobin levels lower than 13.4 gm/dl among home dwelling women 65 and older. Based on these data, the author proposed that the current definition of anemia by the WHO be revisited. These authors also found the risk of dying decreased 0.76 times for every increase of 1 g/dL in hemoglobin between 8 and 12 g/dL. Common complications of anemia are listed in Table 3.

The most common and disabling chronic symptom of cancer and cancer treatment, [18, 86, 122] fatigue is particularly common after age 65 [18]. In these subjects, it may lead to progressive functional decline, delayed cancer treatment, suboptimal cancer control and substantial increase in the cost of managing these patients [86 18, 122]. Even in the absence of cancer anemia has been associated with functional decline among older individuals [15-17]. Of special interest, in the study of Ferrucci et al, an inverse correlation between hemoglobin and function was present also for hemoglobin levels above 12 gm/dl[16].

In patients with chronic renal failure anemia was associated with increased prevalence of congestive heart failure and coronary deaths, [123-126] neurologic symptoms and cognitive decline, [127-130] and correction of anemia

prevented or reversed these complications. A recent review of Medicare patients admitted to a coronary care units showed that a hematocrit lower than 33% (grossly corresponding to hemoglobin levels of 10 gm/dl) was associated with enhanced risk of coronary deaths [126].

Anemia may increase the risk of adverse drug reaction, by a reduction of percentage of drugs bound to red blood cells and increased concentration of free drug in the circulation and by hypoxia that increases the susceptibility of these tissues to therapeutic complications [131]. In post-operative hospitalized patients over 70, anemia was associated with increased risk of delirium [130]. At least four studies showed that the risk of complications of cytotoxic chemotherapy, especially myelosuppression, increases in the presence of anemia[7-10].

3.4 Management of Anemia

Clearly anemia has a number of complications that may be particularly deleterious to older individuals and appear preventable with the correction of anemia. The treatment of underlying causes is the mainstay of management. In some forms of anemia of chronic diseases such as cancer and rheumatoid arthritis, correction of anemia resulted in improved quality of life and function and possibly in improved survival [103-107]. Ongoing studies explore the treatment of all forms of anemia of chronic diseases with erythropoietin in older individuals. It is tantalizing to hypothesize that the correction of anemia may result in improved function, cognition, lesser co-morbidity and possibly improved survival and active life-expectancy.

4. AGE AND THERAPEUTIC EFFECTIVENESS OF HEMATOPOIETIC GROWTH FACTORS

The effectiveness of filgrastim, sarmograstin and erythropoietin in therapeutic doses is well established in older individuals.

4.1 Myelopoietic Growth Factors

A number of studies documented the effectiveness of these substances in older individuals. A retrospective study of the English literature until 1991 demonstrated that the effectiveness of filgrastim and sarmograstim was similar in individuals younger than 65 and those older [132]. In healthy volunteers aged 70 and older, filgrastim induced the same increment in neutrophil count and neutrophil mitotic pool as seen in younger

individuals [133]. In four randomized controlled trials (Table 3), filgrastim reduced the incidence of grade four neutropenia and neutropenic infections for patients with large cell lymphoma aged 70 and older[34, 39,134,135].

In Acute Myelogenous Leukemia both sargromostin and filgrastim reduced the risk of neutropenic infections and the duration of neutropenia in patients aged 60 and older [84, 136-139]. From this review there is definitive evidence of effectiveness for filgrastim, whereas the effectiveness of sarmograstin is suggested only in AML. The recent introduction of PEG-filgrastim requiring only one injection after chemotherapy appears particularly beneficial to older individuals both in terms of convenience and cost.

4.2 Erythropoietin

In patients of all ages, erythropoietin relieves anemia associated with renal insufficiency, [140] anemia of chronic diseases [115], cancer- and chemotherapy- related anemia [103-107]. Prophylactic use of erythropoietin in women receiving adjuvant chemotherapy for breast cancer reduced the risk of anemia and fatigue in a randomized-controlled study [141]. Improvement of anemia was associated with improved energy levels in patients receiving cytotoxic chemotherapy, and the highest incremental energy improvement was obtained when hemoglobin rose from 11 to 13 gm/dl [105, 107]. Erythropoietin α may be administered weekly [105], whereas the new compound, darbepoietin α may be administered every two or every three weeks.

Of special interest, recent studies both in experimental animals and in humans suggested that erythropoietin α may protect the brain from different forms of injuries, including ischemia, degenerative disease and toxins (it is not clear whether this effect is independent from the correction of anemia) [142].

Erythropoietin appears to hold a number of important promises in the management of older patients with and without cancer, to be tested in randomized controlled studies. These include improved function by reversing anemia of chronic disease, prevention of fatigue and functional dependence in older individuals receiving cancer chemotherapy with prophylactic erythropoietin, and preservation of cognitive function in older individuals treated with chemotherapy.

4.3 Other Hemopoietic Cytokines

Data on the effectiveness of growth factors for megakaryocytes are very limited. The activity of Interleukin-11 (oprevelkin) does not appear age-related[70]. Clincal trials with recombinant megakaryocytic growth factors are ongoing[71].

4.4 Recommendations for the Treatment of Elderly Cancer Patients with Hemopoietic Growth Factors

Clearly age is a risk factor for chemotherapy-induced myelotoxicity. Based on the evidence reviewed so far it is reasonable to recommend that Filgrastim or pegfilgrastim be used prophylacticly in older individuals receiving moderately toxic chemotherapy (CHOP and CHOP-like regimens). The hemoglobin of these patients should be maintained at levels of 12 gm/dl or higher for the duration of chemotherapy.

These recommendations were originally formulated for individuals aged 70 and over, [143] and were extended by the American Society of Clinical Oncology to individuals aged 65 and older [144]. Though these recommendations appear to increase the cost of treatment the opposite may well be true. Recent studies of cost-effectiveness demonstrated that the prophylactic use of filgrastim reduces the overall cost of treatment when the risk of neutropenic infection after the first course of treatment is 20% or higher [145]. This threshold is lower than the risk of neutropenic infections in older lymphoma patients receiving CHOP [33-39, 81, 82]. Furthermore, the duration of hospitalization for neutropenic infection is 30% longer in persons aged 65 and over, which implies a higher cost of managing this complication and a higher cost-effectiveness for its prevention [38]. Last but not least, prolonged hospitalization is a risk factor for deconditioning in older individuals, which may involve decreased treatment tolerance, prolonged and costly rehabilitation as well as the need of costly home care and home assistance.

The study of the cost of anemia is more complex, but it appears reasonable to assume that the management of this complication should not substantially affect the total cost of management for the following reasons:

- A recent study demonstrated that the cost of a monthly treatment with erythropoietin is comparable to the cost of two monthly blood transfusions (145).
- Among cancer patients the cost of fatigue is substantial (146).
- Fatigue may reduce the working capacity of as many as 50% of the cancer patients and 25% of their caregivers. Fatigue may precipitate

functional dependence in older individuals (15-18,86), with two costly consequences. First the patient may become incapacitated to provide important money saving functions, such as caregiving for an older spouse or for the grandchildren. Second, the patient himself/herself may need a home caregiver as well as costly rehabilitation.

5. CONCLUSIONS

Aging appears associated with a progressive reduction in hemopoietic reserve due to exhaustion of pluripotent stem cells, increased circulation of catabolic cytokines, and possibly alterations in the microenvironment and in the production of hemopoietic growth factors. In many respects hemopoiesis may reflect general age-related changes.
Whereas hemopoiesis is adequate to maintain the homeostasis of the peripheral blood, it may fail in presence of hemopoietic stress. This event is documented by increased incidence and prevalence of anemia with aging and increased risk of mielodepression following cytotoxic chemotherapy.

In older individuals anemia is associated with increased risk of death, cardiovascular diseases, pharmacologic complications, dementia and functional dependence.

Filgrastim, pegfilgrastim, erythropoietin α and darbepoietin α are effective in older individuals, may prevent complications of cytotoxic chemotherapy, such as neutropenic infections and fatigue, and may lead to cost savings.

The current ASCO guidelines recommend that prophylactic filgrastim or pegfilgrastim be used in patients aged 65 and older who receive moderately toxic chemotherapy and that the hemoglobin of these patients be maintained at 12 gm/dl or higher.

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Chapter 7

CLINICAL AND BIOCHEMICAL EVALUATION CHANGES OVER AGING

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Aging is associated with susceptibility and reduced ability to respond to internal and external stressors. A reduction of functional reserve occurs in many physiological systems and determines increased vulnerability to diseases and high risk of functional dependence. While these modifications can be observed in most persons, particularly in the context of longitudinal studies, they are characterized by extreme variability across individuals and only modest synchronism with chronological aging. As a result, the degree of susceptibility to stressors and exhaustion of functional reserve is dispersed over a wide spectrum in persons of the same age, and the amount of dispersion becomes even greater when we consider older age groups.

By convention, geriatricians define “frail” as those individuals that are at the extreme edge of the severity spectrum in this process. Because of this conventional attitude, frailty is often used as an exchangeable term for disability, comorbidity and poor health status, and it is also considered as an

irreversible condition leading to adverse health outcomes. On the contrary, it is important to conceptualize "frailty" as a continuous, other than a discrete process, where several stages or degrees of severity can be defined as they become useful in research and clinical practice, and that below a certain degree of severity it can be reversed with appropriate interventions.

The aging process is probably associated with the development of some unavoidable degree of "frailty" which in the literature is often referred to as "normal aging". Even the healthiest octogenarian is more sensitive to the effects of stressors than the healthiest teenager. Diseases and behavioral risk factors contribute to frailty in ways cannot be completely explained by traditional biomedical expectations. The effect of environment on frailty is complex. An environment that is too challenging causes a rapid exhaustion of functional reserves and leads to an overt instability of the biological homeostasis. On the other hand, an environment that is not at all challenging, leads to a progressive "atrophy" of the homeostatic mechanisms and makes the individual more susceptible to future stressors.

Clinicians who care for older patients face several times daily the complex implications and questions posed by the age-associated frailty. For example, it is clearly difficult to prescribe a pharmacological treatment, make decisions about rehabilitation, give advice to patients about the risk/benefits of a specific surgical procedure or establish the prognosis of diseases, without having information regarding the degree of functional reserve and ability to respond to stress. On the other hand, there is still much disagreement and discussion of the criteria that should be used for the operational definition of frailty, and most have no idea as to how to grade its severity.

There is some consensus that the basic clinical features of the frailty syndrome should include the following domains: a) mobility, such as lower extremity performance and gait abnormalities; b) muscle weakness; c) poor exercise tolerance; d) unstable balance; e) factors related to body composition, such as malnutrition, and sarcopenia (loss of lean body mass), and weight loss. Validity of these factors as critical elements of the frailty syndrome is provided by studies showing that in older, non-disabled persons, individual components are associated with the classical geriatric syndromes (e.g. falls, symptomatic depression, urinary incontinence and functional impairment) and are strong and independent risk factors of disability and death.

In 1999, Walston and Fried [1] developed an interpretive framework that combines the elements of the "body composition" and "mobility" domains of the frailty syndrome into a pathophysiologic pathway where sarcopenia and poor muscle strength, by limiting mobility and physical activity, reduce total energy expenditure and nutritional intake, which, in turn, lead to weight loss and further aggravate sarcopenia. Using data from

the Cardiovascular Health Study the elements of the pathway were as follows: 1) unexplained weight loss; 2) poor grip strength; 3) self-reported exhaustion; 4) slow walking speed; and 5) low physical activity. After adjusting for significant confounders, participants with 3 or more of these characteristics were at significantly increased risk of disability, hospitalization and death. The work of Walston and Fried [1] demonstrates that aggregating measures in the domains of physical function and body composition are an effective initial basis for developing screening criteria for an intrinsic vulnerability that have predictive validity. However, without understanding the pathophysiologic pathway that leads to frailty as a syndrome that justifies the aggregation of the domains proposed by Waltson and Fried [1], we lack the critical information to envision any serious attempts to apply the concept of frailty into clinical practice. In this chapter we explore some of the biological mechanisms that tend to become dysregulated with aging and may contribute to the pathophysiology of the frailty syndrome. Some of this information concerns biological markers of frailty that can be already measured. So the possible use of these measures in current clinical practice are pointed out in the various sections of this chapter. As our understanding of the pathophysiology, clinical presentation, and consequences of the frailty syndrome improves, many additional uses of the measures will emerge, and will help identify new potential targets for intervention.

1. HUMAN AGING

Gradual physiological changes that often parallel the aging process contribute to the conventional view of “normal” aging. Normal aging implies a progressive decline of the physiological reserve and the ability to compensate, but it is compatible with autonomy over the entire life span. In frail, older persons the decline in functional reserve is accelerated and compensatory mechanisms start failing with consequent negative health outcomes as the functional reserves are depleted.

A better understanding of physiologic changes that proceed and accompany frailty and, over time, lead to disability is needed if we want to capture this pathological process in an early stage, and develop targeted interventions that will delay or postpone the onset of disability. Unfortunately, we have very little information on this topic and, worse yet, what is known is sparse and difficult to reconnect to an overall paradigm. This chapter attempts to address this problem. We will focus on body composition changes, chronic inflammation, oxidative stress and hormonal changes that often occur in older persons and are accelerated over the aging process. Additionally, in the final part of our discussion, we provide our

view on how this information can be used in clinical practice to provide better care to frail older persons.

2. OVERVIEW OF BIOCHEMICAL MARKERS AND AGING (OR FRAILTY)

Many efforts have been made to identify biochemical markers of aging in both normal and frail older individuals [2,3]. Traditionally, clinical chemistry results obtained from laboratory testing are compared with the corresponding reference values in order to determine whether such values fall within the central 95% area under the Gaussian symmetric bell-shaped curve, or the "normal range". Reference values calculated using this method are reported, for example, in the recommendations of the Expert Panel on Theory of Reference Values of the International Federation of Clinical Chemistry and the published guidelines of the National Committee for Clinical Laboratory Standards [4]. In spite of this generalized trend, several lines of research indicate that a purely statistical approach to the identification of "normal" values can be misleading, and methods based on predictive validity in relation to health outcomes should be explored. This is particularly evident for reference values in geriatric patients. Tietz et al. [5] obtained data from 236 individuals, ages 60 to 90 years, 22 individuals, ages 90 to 99 years, and 69, 100 years of age or older. As shown in Table 1 (Tietz et al), plasma levels of dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) were lower in individuals over the age of 90 compared to those of young adults. Other sex hormones, estradiol, estrone and testosterone were found to be much lower in persons over the age of 90 than those of young adults. Interestingly, insulin levels tended to increase in adults aging from 60 to 90 years of age, while a decline in insulin levels was observed in persons over the age of 90.* (Table 1)

The need for biological markers of pathology in the evaluation of older persons is justified by the peculiar relationship that exists between diseases and health status in old age. Because aging is associated with an increment in the global susceptibility to diseases, multiple morbidities are very common. Analogously, diseases that are not clinically overt are often associated with pathological processes that already affect the health status but have not reached the severity threshold that makes them identifiable as "diseases". Thus, the global burden of comorbidity can be captured only indirectly, using functional or biological markers.

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* Note that age-adjusted "normal" values for most of these hormones are lacking and the values prepared in the literature are highly variable from author to author.

Table 1. Laboratory ranges (95th percentile) and mean values of some hormones in young adults, 60- to 90-year olds, and > 90 years of age

		Young adults		60-90		>90	
Analyte	Sex	Range	Mean	Range	Mean	Range	Mean
DHEA, mg/L	M + F	1.60-8.00				0.17-1.69	0.79
DHEAS, mg/L	M	1800-4500				40-750	287
	F	1200-3150				20-600	231
Estradiol, ng/L	F	Follicular:30-100 Luteal:70-300				<5-20	6
Estrone, ng/L	F	Follicular: 30-100 Luteal: 60-160				<5-58	29
Insulin (mU/L)	M+F	6-23	11.8	6.6-36.7	16.4	2.4-19.0	7.20
Testosterone total (mg/L)	M	3.50-10.30				2.15-6.71	3.40

As reported by Tietz et al. (5)

Impaired glucose, protein and lipid metabolism in older individuals is common. Aging is associated with impaired glucose handling, mainly due to a decline in insulin activity [6-8]. There is strong evidence that increased resistance to insulin activity is one of the main components of diminished homeostatic glucose regulation in older persons. Insulin-mediated glucose uptake, measured using a glucose clamp technique combined with H^3-glucose infusion, was shown to progressively decline over aging [9]. The response to insulin resistance is increased insulin production. When the ability to compensate for insulin resistance by increasing insulin production is exhausted, the glucose homeostasis becomes dysregulated, and type 2 Diabetes Mellitus occurs. Therefore, insulin resistance along with the reduced ability to secrete insulin, as seen during glucose tolerance test administration in elderly subjects, also contributes to impaired glucose homeostasis [10,11]. However, the dysregulation in the glucose metabolism due to peripheral insulin resistance is important long before diabetes can be

diagnosed and cannot be overlooked in the clinical evaluation of older persons.

Total body protein, lean body mass and the rates of protein synthesis decline with increasing age. More importantly, such changes are components of an impaired homeostatic phenomenon, which is not always balanced with adequate dietary protein intake. Furthermore, an altered state of hepatic protein synthesis with reduced fibrinogen and other protein carriers, such as thyroxine-binding protein and iron-binding protein may result in an altered coagulatory state, reduced thyroxine plasma concentrations and an anemic state. In addition, reduced plasma concentrations of albumin have been correlated with a higher degree of oxidative stress.

Lipid and lipoprotein concentrations vary over an individual's lifespan [12]. In particular, total cholesterol and triglyceride levels tend to increase up until 50 years of age and then a gradual decline starts to occur. Interestingly, a positive correlation exists between total cholesterol and/or triglyceride levels with the incidence of cardiovascular disease up to the age of 50 years. However, the ability of total cholesterol to predict coronary heart disease in very old individuals remains controversial. Raiha et al [13] reported that an elevated level of total cholesterol was not a cardiovascular risk in older persons, but predicted survival for non-cardiovascular disease mortality, while Manolio et al [14] did not find any correlation between total cholesterol and all-cause mortality in older subjects [13]. Interestingly, studies have reported that persistently low cholesterol levels increased the risk of mortality in males aged 71 to 93 [15]. Low total cholesterol levels have also been associated with all-cause mortality in elderly Italian men and women, thus underscoring the potential importance of low levels of cholesterol as a warning sign of rapidly declining health [16].

Such discrepancies can be explained by one of the major differences between middle-aged and older populations, which is the presence of an increased prevalence of poorer health of older individuals. In fact, older frail persons with low total cholesterol levels are more likely to have a decreased survival rate than older persons with little or no disease in the presence of chronically low cholesterol values [17]. Interestingly, after adjusting for frailty markers in a large sample of older persons, elevated total cholesterol levels predicted an increased risk for death from CHD, and the risk of death from CHD decreased as cholesterol levels declined [18]. These authors also emphasized the finding that frailty markers were consistently associated with low cholesterol levels, thus confirming similar previous reports. Only further investigations aimed at evaluating controlled clinical trials with lipid-lowering therapy in non-frail older persons can shed light on the risks or the benefits of such treatment.

Regarding lipoproteins, high-density lipoprotein cholesterol (HDL-C) is a considered a protective factor for CHD [19]. In particular, HDL-C levels

have been associated with good health status, while reduced HDL-C values are recognized as risk factors for CHD in both middle-aged and older persons. Furthermore, it has been shown that reduced HDL-C also predicts non-CHD/stroke mortality in older persons [20]. Thus, low HDL-C may also be considered a valid biomarker for chronic disease and poor health status in old age.

Ueno et al[3] have recently described biomarkers of aging in women. In particular, these authors suggest that five variables should be considered specific biomarkers for aging in women: forced expiratory volume in 1.0 s (FEV_1), systolic blood pressure (SBP), glucose (GLU, mg/dl), ratio of albumin to globulin (A/G) and mean corpuscular hemoglobin (MCH, pg). Such multiple physiological variables reflect the function of diverse vital functions, in particular, pulmonary function, blood pressure, glucose handling, protein metabolism and hematological functioning. Biological age scores (BAS) were calculated using the parameters mentioned above. Ueno et al [3] concluded that the rate at which women age is relatively slow up until 65 years of age. Then after 65 years of age their rates of aging rapidly increase. Therefore, the biological processes aimed at maintaining a stable homeostasis correctly function up to age 65; after 65, false signaling of such a complex system occurs causing it to lose its effectiveness. This observation is of extreme importance as altered biomarkers are highly correlated with mortality [21] and the frailty syndrome is commonly observed in persons over the age of 65.

The aggregation of variables in global indices based on their predictive role for specific outcomes is very appealing for clinical use, but adds very little to our understanding of the global burden of disease in old age. Recently, authors have also suggested that the involvement in multiple physiological systems that is characteristic of older patients with comorbidity should be interpreted in the context of the "frailty syndrome". According to current views of frailty, homeostasis is disrupted when the ability of individuals to respond to internal and external changes declines below the threshold of effective compensation. When this occurs, abnormal concentrations of specific biomarkers of frailty become detectable in the biological fluids, and structural changes take place in cells and tissues. Unfortunately, serum biomarkers are not currently used to identify frailty, which still remains a clinical diagnosis based on medical history, symptoms, and signs. Clinically, the frailty syndrome is characterized by an excessive reduction in lean body mass, in walking performance and in endurance, associated with a perception of exhaustion and fatigue [22]. Several lines of evidence, however, show that this syndrome is often paralleled by important changes in physiological systems accompanied by changes in serum levels of biomarkers.

3. FRAILTY AND THE NEUROMUSCULAR SYSTEM

There is growing evidence that the core target of the frailty syndrome is motor organization, specifically the muscular and nervous systems. Disease, disuse and aging trigger a mechanism that impoverishes the redundancy of muscular and nervous backup systems, leading to a measurable decline of motor performance. Once the process is activated, its consequences follow a common pathway leading to a more generalized loss of motor functioning. There is good evidence that measures that are related to mobility and motor performance are interpretable as proxy markers of frailty. However, the "diagnosis" of frailty, as a syndrome, hides an array of different pathologic processes that may involve the integrity and functionality of selected physiological subsystems implicated in motor performance [2]. Some of these subsystems include: bone, joints, muscles, peripheral nerves, metabolic efficiency, aerobic capacity and energy production. Clinically, the best criteria for screening of frailty are tests of mobility, gait, balance, manual dexterity, activities of daily living (ADLs) [23], instrumental activities of daily living (IADL) (24) and the Barthel Index [25]. However, it is conceivable that specific biomarkers could be measured in order to identify the involvement of each one of these physiological systems in the early stages of the disablement process.

Lower extremity performance in non-disabled persons is an excellent predictor of poor quality of life, deterioration of health status, incident disability, health care utilization, nursing home admission and death. Thus, physical performance measures have been considered "vital signs" of functional decline in older persons [26]. In particular, gait speed and the short performance battery, developed in the context of the EPESE study, have been identified as quantitative estimates of future risk for functional decline and hospitalization [26] Observational studies provide good evidence that performance-based measures of mobility are valid proxy measures of frailty and global susceptibility to adverse health outcomes.

In older persons, poor muscle strength and poor physical performance often coexist. Midlife handgrip muscle strength has been recognized as an important factor that predicts old age functional ability [27]. Observational studies have consistently shown that chronic conditions such as coronary heart disease, diabetes and pulmonary obstructive disease are associated with lower muscle strength. These findings suggest that a core mechanism exists that is responsible for changes in body composition and disease susceptibility in old age and ultimately to the age-associated changes in functional capacity. Possible links between diseases in old age and "frailty" are: nutritional depletion, inflammation, reduced physical activity or inactivity. These mechanisms are, in turn, risk factors for mortality. Thus,

in persons afflicted with chronic illness, reduced muscle strength could be considered an important marker of disease severity. Indeed, handgrip muscle strength has also been associated with overall mortality, independently of poor nutritional status, inflammation and physical inactivity [28]. These findings suggest that muscle strength has a direct effect on mortality or increases the risk of mortality through a mechanism that is still unclear.

4. BODY COMPOSITION CHANGES

The two main components of body composition are fat mass and lean (fat-free) mass. Fat-free mass consists of body cell mass, extracellular fluid and the extracellular solids such as collagen and bone mineral [29]. The body cell mass may be further subdivided into the fat-free portion of cells within muscle, viscera and the immune system. The body cell mass is functionally the most important compartment in determining energy production and expenditure, protein needs, and metabolic response to stress (acute phase response).

There are substantial changes in body composition that accompany the aging process [30]. In particular, the fat mass increases and accumulates preferentially in the abdominal area, while a parallel decline in muscle mass and bone density occurs. Interestingly, the changes in body composition that begin to manifest during adulthood may be partially explained by an imbalance of energy intake and expenditure. In older adults, however, these changes are extremely accelerated compared to younger cohorts, and cannot be explained simply as an imbalance between energy intake and expenditure.

In most older persons, fat mass constitutes a greater percentage of total weight than individuals at younger ages. A population-based study in which anthropometric parameters were measured over the entire life span (age range: 20-103 yrs.) demonstrated that the accumulation of abdominal fat with age occurs primarily during middle age [31] but is different for men and women. In particular, the greatest change of waist circumference seems to occur in men between 20 and 55 years of age, while in women, the waist circumference tends to increase progressively across the entire life span.

Many studies have shown that increased visceral fat is a risk factor for age-related diseases such as hypertension, type 2 diabetes, cardiovascular disease and some types of cancer [20,32]. Adipose tissue has also been correlated with oxidative stress, reduced glucose uptake, and reduced insulin clearance. Understanding how changes in body composition and, in particular, fat distribution, affect the risk for many disease states and mortality is one of the most important research questions that should be addressed in future studies.

The simplest clinical indicator of visceral fat is the waist circumference. A number of studies have shown that waist circumference is an independent risk factor for cardiovascular disease in adults, including those 65 years and older. On the contrary, the relationship between body mass index (BMI), cardiovascular disease and all-cause mortality is controversial. The highest mortality rates have been found in older persons with very low BMI, while in middle-aged persons, BMI was positively associated with mortality [33]. These data suggest that the relationship between body composition and health-related outcomes in older persons cannot be evaluated simply in conventional terms of body fat, but rather fat distribution and type of fat accumulated, both providing essential information for assessing such risk.

The notion that aging is associated with gradual reduction of lean body mass is also generic. In fact, selected tissues seem to be more affected by aging than others. In particular, the decline in non-fat mass is largely attributed to sarcopenia. Sarcopenia has been increasingly used to describe the age-related decline in both muscle mass and muscle strength. However, despite the term "sarcopenia", the precise criteria that define such a state have still not been agreed upon.

Changes in body composition that parallel the aging process are strongly associated with a decline in physical function and mortality risk. The underlying mechanisms responsible for the excess age-associated decline of muscle mass and function compared to other sections of lean body mass are still unknown. Several hypotheses have been proposed, which include: i) intrinsic biochemical and physical changes leading to muscle atrophy [34]; ii) reduced neuronal stimulation due to reduction in the number of α-motorneurons or their activity [35]; iii) oxidative damage of mitochondrial DNA with accumulations of mutations that reduce the efficiency of the metabolic pathways aimed at energy production [36,37]; iv) influence of external factors such as malnutrition, sedentary life-style and disease typically observed in older persons (38); v) loss of endogenous hormone production [39,40]; and vi) dysregulation of catabolic cytokines [41,42]. Over the aging process, both changes in the contractile efficiency of muscle fibers and changes in tissue quality, such as an increase in connective tissue and pericellular fat infiltration, may also contribute to altered muscle function.

There are many methods that simultaneously measure body fat and fat-free components of body composition. Some important measures are as follows: 1) skin-fold measurements are obtained using hand-held calipers, which exert a standardized pressure at various body locations. The sum of these measurements is used to derive body fat percentage. The caliper method is based on the idea that the thickness of subcutaneous fat reflects a constant proportion of the total body fat and that the sites selected for measurements represent the average thickness of the subcutaneous fat [43,44].

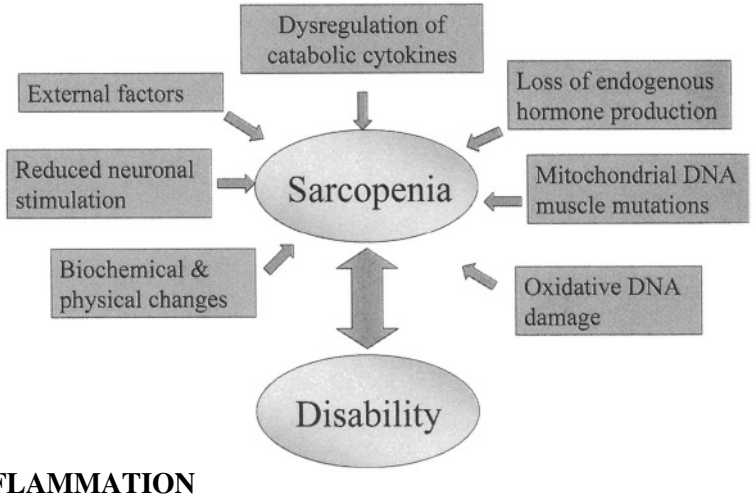
Also, the mean arm circumference with triceps skin-fold thickness is used to calculate muscle area and thus, derive fat-free mass; 2) hydrodensitometry or underwater weighing is another instrumental method that measures whole body density by determining body volume. This technique is based on the two-compartment model (fat and fat-free mass). The densities of bone and muscle are higher than water. Body fat percentage is then calculated from body density using standard equations (sirk or brozek); 3) bioelectrical impedance analysis (BIA) is measured utilizing a safe electrical signal that passes through the body. Impedance is greatest in fat tissue (10-20% water), while fat-free mass (70-75% water) allows the signal to pass much more easily. The measurement obtained is entered into a formula along with height, weight, and gender to determine lean and fat mass. However, in order to obtain correct evaluations from the BIA it is necessary that the body is within normal hydration ranges.

Newer more sophisticated methods for the assessment of body composition include the Dual Energy X-ray Absorptiometer (DEXA), computed tomography (CT), magnetic resonance imaging (MRI) and air displacement plethysmography . DEXA is a relatively new method that uses three compartments (total body mineral, fat-free mass, and fat mass). DEXA consists of a dual energy beam (two low dose x-ray sources) that scans bone and soft tissue simultaneously. DEXA is currently considered the "gold standard" measure because of the high degree of precision in a single measurement and the ability to provide the exact location of fat tissue distribution. CT scanning produces cross-sectional scans of the body. As the beam rotates data is collected, stored and applied to algorithms to build images that describe body composition. MRI utilizes a magnetic field that "excites" water and fat molecules, producing a measurable signal, which is then measured and analyzed. Whole body air displacement plethysmography (trade name BOD POD) is a new technique that is similar to the underwater method, but uses air displacement instead of water. It is based on Boyle's law, which states that volume and pressure are inversely related. All the methods described above are summarized in Table 2 (shown on page 154), with information on their reliability, advantages and disadvantages.

It is interesting to speculate on the consequences of sarcopenia that are not directly related to poor muscle strength. A number of physiological functions that take place within muscle tissues have a critical effect on human metabolism: muscles are a reservoir of body proteins and energy that can be utilized in periods of extreme stress or malnutrition; amino-acids can be mobilized during acute infections and are used as building blocks for antibodies; hormones are produced and catabolized in muscle tissue. Thus, age-related muscle mass reduction may explain the lower metabolic adaptation and immunological response to disease. Indeed, poor muscle strength is a strong predictor of mortality, independent of any other known

risk factors for poor muscle strength. The rate of decline varies among individuals and is influenced by factors that modulate the balance between catabolic and anabolic processes. There are several possible mechanisms that may be involved in the genesis of sarcopenia (Fig. 1). The most important of these mechanisms are prolonged pro-inflammatory state, change in hormone secretion signaling activity, and unopposed oxidative stress. However, recent data suggest that these three pathophysiological mechanisms are highly interconnected and should be interpreted as components of a unique process leading to frailty and disability in old age [45].

Figure 1. Possible factors involved in the genesis of sarcopenia



5. INFLAMMATION

Increased circulating and tissue levels of inflammatory markers have been observed in older persons, especially those who are frail and/or affected by comorbidity. Normally, cytokines or other biomarkers of inflammation initiate and regulate the acute phase inflammatory response during an infection, a trauma or any other type of stress. However, studies have suggested that a primary dysregulation of the mechanisms that initiate, modulate and shut off an inflammatory response often occurs with aging [46,47]. Such a dysregulation is mainly testimonied by high plasma levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6) (48-50), (Interleukin-1) IL-1 and acute phase proteins in older persons [46]. In extreme situations, such as diseases that cause prolonged hypercatabolic states, severe muscle "wasting" may develop over a short period. However, a certain degree of muscle "decline" has been attributed to the reduced capacity of skeletal muscles to synthesize new proteins in the

aging process [29]. An imbalance between muscle protein synthesis and degradation occurs, ultimately leading to reduced muscle mass, protein content and strength. Such imbalance has been linked to pro-inflammatory cytokines capable of inducing proteolysis or inhibiting protein synthesis. TNF-α induces muscle proteolysis and plays a significant role in muscle wasting (cachexia). TNF-α and IL-6 can also inhibit protein synthesis, either directly or by interfering with IGF-1 signaling[51].

Elevated plasma levels of each of the pro-inflammatory cytokines mentioned above have also been observed in many age-related diseases, such as anemia, osteoporosis, sarcopenia, atherosclerosis, cancer, type 2 DM, impaired cognitive functioning, and Alzheimer's disease. This further supports the theory that a core mechanism contributes to overall age-associated changes in functional capacity.

6. HORMONES

Anabolic agents shift the anabolic/catabolic balance of protein metabolism toward the synthesis of new proteins, which is needed to replace the proteins that are continuously catabolized, therefore maintaining muscle integrity and volume. Hypertrophy requires the proliferation of muscle nuclei (hyperplasia) in order to maintain the nuclear/cytoplasmic ratio (52). Hormonal factors shown to be related to muscle hypertrophy are: Insulin-like Growth Factor-1 (IGF-1), Growth Hormone (GH), testosterone and dehydroepiandrosterone. High IGF-1 concentrations are associated with characteristics that are opposite to those typical of aging, including decreased body fat content, increased muscle mass and improved metabolic homeostasis of glucose and lipids. At the muscular level, IGF-1 stimulates protein synthesis and satellite cell differentiation, thus, playing a crucial role in the maintenance of muscle mass and function. Many studies have provided insight into the signaling pathways by which IGF-1 affects muscle anatomy and function [53-55]. Circulating IGF-1 concentrations decrease with advancing age. The age-associated decline in IGF-1 plasma concentrations is influenced by reduced GH levels, and also by nutritional status, insulin and inflammatory cytokines. Specifically, the biologic activity of IGF-1 on muscle strength can be inhibited by IL-6 [55], suggesting that the detrimental effect of inflammation on muscle functioning may be mediated by IGF-1. Furthermore, studies provide evidence that the higher concentrations of pro-inflammatory cytokines found in older persons directly interferes with the IGF-1 gene protein expression and receptor sensibility in muscles [55,56]. High IL-6 and low IGF-1 plasma concentrations are considered risk factors for poor muscle strength, poor lower extremity performance and disability.

The aging process is associated with the loss of many anabolic signals to muscle function. Recent studies have shown that age is not only accompanied by a decline in anabolic activity, but an increase in catabolic signals as well. In fact, impairment of the anabolic IGF-1 signaling pathway may have several negative effects:

1) Reduced physical activity that is often observed in advanced age causes decreased stretch-activation stimulation of different muscle isoforms of IGF-1;
2) An age-related decline of GH influences IGF-1 muscle response;
3) The progressive loss of appetite with reduced food intake can result in malnutrition and eventual "wasting";
4) Loss of motoneurons that are essential for skeletal muscle functioning leads to atrophy and increased proteolysis.

There is evidence that the age-associated decline in GH levels in combination with lower IGF-1 levels also contributes to the development of sarcopenia [57,58]. The reduced pituitary secretion of GH is probably due to age-related changes in the GH-releasing hormone (GHRH). Unfortunately, treatment with GH has demonstrated many adverse effects, such as peripheral edema, arthralgias, glucose intolerance and type 2 diabetes [40]. Investigations have demonstrated that therapy with GHRH (somatorelin) in older persons is capable of restoring the age-related decline of the GH response [59]. More studies attempting to verify whether such pharmacological approaches can restore muscle functioning as well as the metabolic homeostasis in elderly persons while minimizing side effects are underway.

Testosterone affects muscle mass and muscle strength both directly and indirectly. It has been reported that testosterone increases protein synthesis and intramuscular mRNA concentrations of IGF-1 and decreases inhibitory IGF binding protein 4 concentrations [60]. Due to evidence that testosterone levels decline with advancing age, a negative impact on muscle function is not surprising. Older men with low circulating levels of testosterone tend to have lower muscle strength than men of the same age with normal testosterone, and studies utilizing supplemental therapy with testosterone have shown an increase in muscle mass and strength in elderly males. Testosterone has also been linked to body composition changes such as an increase in muscle mass and a decrease in fat mass [61]. The widespread use of testosterone replacement remains controversial due to safety concerns and inconsistent reports regarding clinically important outcome measures.

The production and the circulating levels of adrenal sex hormone precursors, dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS), decline significantly with aging [62]. DHEAS serum levels have been correlated with parameters of body composition. Some clinical trials have shown that supplementation with DHEA resulted in increased muscle strength and decreased body fat[63]. However, more recently these findings

were not confirmed in a large randomised controlled trial performed in men 60 to 80 years old. The mechanism by which DHEA acts on muscle function is probably related to the peripheral conversion to testosterone and dihydrotestosterone, but a direct effect of DHEAS cannot be excluded since specific receptors have been identified in muscle tissue.

Estrogen levels also decline with aging. Although estrogen has a direct anabolic effect on muscle cells in vitro, several authors believe that the effect of estrogen on muscle is mediated by their conversion to testosterone [64]. Interestingly, both estrogen and testosterone are capable of inhibiting IL-6 production, suggesting that an age-related decline of such hormones would play a pivotal role in catabolic signaling on muscle tissue. However, the available information regarding the effects of supplemental therapy of estrogen on muscle function is limited and the results are inconclusive. While some studies have concluded that estrogen therapy in postmenopausal women does not significantly affect muscle mass or strength [65,66], others suggest that estrogen therapy has a positive effect on body composition. For example, Sorensen et al [67] demonstrated that estrogen replacement therapy was significantly associated with an increase in lean body mass and also a decrease in total body fat.

As previously mentioned, advancing age is associated with impaired glucose handling mainly due to a reduction of insulin peripheral activity. Since insulin plays a pivotal role for muscle contraction by increasing glucose uptake and promoting intracellular glucose metabolism, it is plausible that age-related insulin resistance (IR) may be an important cause of poor muscle strength in old age. Furthermore, a reduction of insulin peripheral activity may reduce the muscle tissue anabolic rate leading to a relative catabolic state and in turn, facilitating sarcopenia. The contraction of Type I fibers is especially dependent on glucose entry and metabolism compared to contraction of Type IIa (fast twitch, oxidative, glycolytic) or IIb (fast twitch, glycolytic) fibers [68]. Type I fibers are more responsive to insulin, and are more representative of the muscle in older persons [69].

Over the aging process, changes in both the contractile efficiency of muscle fibers and changes in tissue quality, such as an increase in connective tissue and pericellular fat infiltration, may contribute to altered muscle function [70]. Moreover, insulin resistance (IR) could be further worsened by the occurrence of pericellular fat accumulation both directly and through the increased production of pro-inflammatory cytokines, such as IL-6 and TNF-α. Furthermore, a recent study demonstrated that a decline in aged skeletal muscle force might also be due to a reduction of L-type calcium channels, resulting in excitation-contraction uncoupling and less Ca^{2+} release by the sarcoplasmic reticulum (SR) [71]. Insulin has a stimulatory effect on intracellular calcium uptake [71]; thus, an age-related state of IR may negatively affect muscle contraction via this mechanism. It is well known

that IGF-1 actively stimulates insulin receptors. Since IGF-1 levels decline throughout aging, the decline in muscle strength that is associated with aging may be mediated by decreasing plasma IGF-1 levels that contribute to IR. Studies will be needed in order to verify if the impact of IR on specific muscle tissue and functioning in aged individuals exists.

Certain changes typically occur in muscles of older adults. The quantity of muscle declines, although this varies between individuals, but the composition of the muscle changes with aging as well. Increased infiltration of fat deposited in skeletal muscle tissue may affect muscular function. Much of the existing data on the association between intramyocellular lipid (IML) content has been obtained directly from muscle tissue biopsies. However, the use of muscle attenuation through computed tomography (CT) scanning, as a measure of IML, has been validated [72]. In 45 men and women, the muscle fiber lipid content determined histologically with oil red staining was correlated with muscle attenuation. Thus, the use of CT-derived muscle attenuation should be considered a non-invasive method of measuring IML. In fact, Visser et al [73] demonstrated that increased skeletal muscle fat infiltration measured by CT scanning was associated with poorer lower extremity performance independently of total body fat and muscle area in older men and women.

7. OXIDATIVE STRESS

The accumulation of lipofuscina [74] and increased cross-linking of collagen [75] were the first observations reported on the effect of the aging process at the cellular level. At that time it was unknown that these modifications are, at least in part, related to oxidative stress. More recently, researchers have focused on the progressive changes that occur in the DNA structure and the underlying causes and potential consequences of these mutations. For example, a number of studies suggest that excess and unopposed oxidative stress is the main cause of increasing mitochondrial DNA (mtDNA) mutations with aging and in several age-related diseases. Accordingly, oxidative stress characterized by an uncontrolled production of free radicals is considered a major factor in the aging process. In aerobic biological systems, free radicals are primarily derived from oxygen and are produced by splitting a covalent bond into atoms or molecules with an unpaired electron, therefore forming highly reactive oxygen species (ROS). In normal physiological conditions, the intra-mitochondrial environment is characterized by a substantial equilibrium between the production of ROS and the activity of anti-oxidant mechanisms, such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Several lines of research suggest that the endogenous production of ROS increases with age and, in

parallel, the activity (but not the tissue concentration) of anti-oxidants declines, therefore increasing the risk of damage due to oxidative stress, especially at the level of the mtDNA. In addition to its effect on mtDNA, oxidative stress also adversely impacts other vulnerable targets, including lipid and protein components of membranes. Free radicals can cause lipid oxidation with a consequent reduction in transmembrane transportation. Age-related overproduction of ROS may also lead to the activation of apoptosis. Therefore, the accumulation of oxidatively damaged mtDNA, together with enhanced apoptosis act synergistically to cause the general decline of biochemical and physiological function of tissues over the aging process. The underlying mechanisms by which these events accompany the aging process remain to be identified and merit further investigation.

Studies suggest that the degree of unopposed oxidative stress is predictive of mortality. In particular, the production of free radicals in the heart, kidney and liver is inversely proportional to the maximum lifespan [76] and rate of mitochondrial oxygen radical generation is negatively associated with animal longevity. In animal models, caloric restriction, which decreases the rate of aging, also decreases mitochondrial oxygen radical production and oxidative damage to mtDNA.

The mitochondrial DNA/oxidative stress hypothesis can explain certain age-related disease states such as Parkinson's disease, Alzheimer's disease and skeletal muscle myopathies. Recently, epidemiological studies have suggested that dietary anti-oxidants may have a significant impact on age-related disease states [77,78]. This remains unproven in clinical trials. The clinical implications of oxidative stress are complex, and intervention studies are needed to further clarify the role of dietary and supplemental antioxidants in the prevention of age-associated frailty.

8. SUCCESSFUL AGING

The possibility of reaching the extreme end of the human lifespan results from the continuous adaptation of the body to respond to negative insults over the aging process. Healthy centenarians are a very selective group of persons representing one of the best living models of "successful aging". Many studies have focused on centenarians' anthropometric, endocrine and metabolic characteristics in order to formulate a clearer clinical picture of successful aging. They report that the average fat free mass (FFM) of healthy centenarians is similar to that of other aged subjects but lower than middle-aged adult subjects [79]. However, most healthy centenarians do not undergo the usual anthropometric derangement found in elderly persons. For example, the waist/hip ratio has been found to be lower in healthy centenarians than in other aged individuals. Regarding endocrine

factors, total plasma IGF-1 concentrations were similar in both healthy centenarians and aged subjects, but the molar ratio IGF-1/IGF binding protein-3, an expression of free plasma IGF-1 concentration, was observed to be significantly elevated in healthy centenarians compared to elderly subjects [80]. This ratio is negatively correlated with body mass index, body fat content, plasma triglycerides, and FFA and LDL concentrations[80].

While serum markers may be useful for helping identify successful aging, caution should be used since the interpretation may be different in younger adults than in older persons. For example, in older persons, the ability of total cholesterol to predict age-related diseases such as coronary heart disease (CHD) has been challenged. In middle-aged adults, total cholesterol levels have been shown to have a direct association with CHD and mortality, but such a relationship in individuals over the age of 65 remains controversial. In older persons, a J or U-shaped association has been reported, suggesting that extremely high or low concentrations have an increased risk of death [81,82]; total cholesterol levels have also been shown to have a positive association, an inverse association, and no association with mortality in older persons.

Up to now, most studies have considered the association of total cholesterol on CHD in subjects under the age of 85 years. Interestingly, a recent study reporting data on fractionated lipoprotein levels among persons over the age of 85 years, concluded that low HDL cholesterol, but not high LDL cholesterol, is a risk factor for mortality from CHD and stroke in persons over the age of 85 [83]. Lipoprotein (a) [Lp(a)], a genetically controlled cholesterol-rich lipoprotein, has been hypothesized as an independent risk factor for premature CHD, stroke, and peripheral artery disease in elderly persons [84,85]. This observation may be due to the presence of Lp(a) in atherosclerotic plaques and its ability to stimulate smooth muscle proliferation [86].

The physiological and pathological roles of Lp(a) probably change with aging. Support for this comes from a study by Baggio et al [87], which reported no significant differences in Lp(a) serum concentrations among healthy centenarians, persons <65 and >65 years of age, even though Lp(a) has been proposed as an independent risk factor for cardiovascular disease. Centenarians with high Lp (a) levels had significantly higher IL-6 levels, thus characterizing the paradox of successful aging. Such data questions the idea that Lp(a) is under strict genetic control and suggests that environmental factors may play a significant role in older adults, including subclinical inflammatory states. Thus, a continuous remodeling of lipid metabolism may occur with aging and may be critical for successful aging. The deleterious reshaping of serum lipids and lipoproteins in young, adult and elderly individuals are considered risk factors for age-related diseases, while their biological significance in healthy centenarians remains unknown. Thus, only

future investigations highlighting age-related changes in lipid physiology of healthy centenarians on mortality rates will resolve such discrepancies.

Healthy centenarians have a lower degree of oxidative stress. In fact, it has been shown that healthy centenarians have greater plasma antioxidant defenses than aged individuals. According to the remodeling theory on aging, the body continuously and correctly adapts to deleterious changes over time. As previously mentioned, an age-related up-regulation of the inflammatory response takes place over the aging process. In both sick and healthy elderly individuals, peripheral blood markers of inflammation (albumin, cholesterol, IL-6 and CRP) have been associated with increased risk for mortality. Interestingly, the age-related increase of serum IL-6 levels has been seen in both elderly and centenarian individuals [49,87]. IL-6 dysregulation has been suggested to play a role in the pathogenesis of a variety of age-related diseases, such as diabetes and atherosclerosis [88]. Indeed, healthy centenarians have elevated pro-inflammatory cytokine concentrations, but do not have the same high incidence of most age-related disease states in other elderly persons. Thus, in healthy centenarians such abnormal cytokine levels may reflect a state of subclinical inflammation. The reason why healthy centenarians adapt correctly to such insults remains unknown.

Whether healthy centenarians have some protective genetic factors that can protect against deleterious changes or facilitate the remodeling process remain unknown. Future investigations will be needed in order to provide the necessary answers.

Tables 3 and 4 summarize some of the clinical and biochemical evaluations described in the text above, and that can be used to assess the degree of “successfulness: of the aging process. Note that these are only examples. An exhaustive list would be very large, and out of the scope of this chapter.

Table 2. Diverse Techniques for Body Composition Evaluations

Method	Reliability	Advantages	Disadvantages
Hydrodensitometry	++	Traditional reference method for body composition research	High cost; long test duration (15-60'); difficult for persons who dislike, can't be submersed in water, or have difficulty expelling air from their lungs; reading errors if air remains in lungs; reading may vary due to body hydration.
Skin-fold Measurements	++	Low cost; test duration: 10-20'	Precision depends on the skill of the technician; accuracy depends on sites measured; difficulty in grasping skin-fold of obese; multiple readings are required for accuracy.
BIA	++++	Test duration: 10'; Moderate cost; low to moderate technician skill.	Electrolyte gel can be uncomfortable; accuracy depends on minimal variability caused by body hydration level; measures derived by the equation used.
DEXA	+++++	Test duration: 10-20';subjects only have to lie still; measures fat distribution throughout the body in a single scan; no need to account for air mass in the lungs.	Very high cost.
MRI	++++	Very useful for high quality images for body fat distribution.	Very high cost; requires a highly skilled technician.
CT	++++	Very useful for ratio intra-abdominal fat to extra-abdominal fat.	Very high cost; exposure to radiation
BOD POD	++++	Brief test duration: 20 sec;	Very high cost; limited in availability; requires further testing in order to verify test accuracy in measuring body composition.

Table 3. Summary of diverse techniques described in the text in older persons

Test	Purpose	Clinical Situation	Specific Examples
Glucose Clamp	To assess insulin action and secretion	Impaired glucose handling	Measurement of insulin-mediated glucose uptake and glucose stimulated insulin secretion (11)
Isometric Muscle strength (upper limb)	To assess handgrip muscle strength	Sarcopenia, predictor of disability and mortality	Handheld dynamometer (27)
Isometric Muscle strength (lower limb)	To assess lower extremity muscle strength	Sarcopenia	Hip Flexion, Knee Extension, Ankle dorsal flexion, Hip abduction
Balance	To assess static balance	Screening for falls	FICSIT balance score (89)
ADL	To assess self-care, mobility and incontinence	Disability screening	Katz (ADL) (23) Barthel Index (25)
IADL	To assess the ability to shop, cook, perform household activities and finances	Disability screening	Lawton (IADL) (24)
Physical performance tests	Quantitatively assess gait, balance, and risk of falls	Valid proxy for frailty and global susceptibility to adverse health outcomes	Tinetti performance oriented mobility (90) assessment Short physical performance battery (91)

Table 4. Summary of some laboratory tests utilized in older people

Test	Values	Clinical Importance
Fasting glucose (mg/dl)	≥126	Diabetes mellitus
OGTT 2 hr glucose (mg/dl)	≥200	Diabetes mellitus
Fasting glucose (mg/dl) +	<110 + <140	Normal glucose homeostasis
OGTT 2 hr glucose (mg/dl)	110-125 + <140	Impaired fasting glucose (IFG)
	<110 + 140-199	Impaired glucose tolerance (IGT)
	110-125 + 140-199	Combined IFG + IGT
Total cholesterol (mg/dl)		
	>200	Hypercholestolemia with increased cardiovascular risk
	<50	Frailty marker
HDL	≤ 35	Risk of atherosclerosis
	≥160	Risk of atherosclerosis
LDL		
Albumin (g/dl)	<3.5	Malnutrition
C-reactive protein	>0.5mg/dl	Inflammatory state
Hemoglobin	men: <12g/dl	Anemic state
	women: <13g/dl	Anemic state
Red blood cells	men: <4.3 x 10^6	Anemia, hemorrhage, hemolysis
	women: <4.0 x 106	
	men: >5.5 x 10^6/µl	Polycythemia
	women: >5.1 x 106/µl	
White blood cells	neutrophils: >7500/µl	Infection, inflammatory state, neoplasms, metabolic disease states
	eosinphils: >500/µl	allergies, neoplasms,
	monocytes: >1000/µl	infection, sarcoidosis
	lymphocytosis: >4000/µl	mieloproliferative disorders
	neutrophils:<1500/µl	neutropenia: altered production, excessiva destruction
	lymphocytes: <1000/µl	lymphopenia: altered immune response

8. CONCLUSIONS

It is widely recognized that the assessment of diseases status performed according to the traditional dichotomy "no disease vs. disease" is insufficient to understand the complexity of problems that influence health and well being in older persons. This concept was recognized long ago by geriatricians and implemented in the paradigm of "Comprehensive Geriatric Assessment". Accordingly, many researchers and clinicians have proposed that the direct assessment of physical and cognitive function provides the essential information that is needed to design effective interventions in frail older persons. However, this approach has never been completely translated

into clinical practice and many geriatricians claim that the administration of any available medical treatment is still conditioned to a previous diagnosis of specific diseases and hypotheses about specific pathophysiological pathways. Furthermore, significant changes in health status may occur and be amenable to effective treatment long before any clear effect on physical and cognitive function is detected.

We propose that the concept of frailty – a condition that involves impairment in multiple physiological systems and is characterized by exhaustion of functional reserve, massive use of compensatory strategies and high risk of homeostatic breakdown – can be used by clinicians to gain a better understanding of the global burden of disease and reduced physical function in older persons and their interaction with the "pure" effect of aging. Unfortunately, there is still no agreement on the criteria that should be used in order to identify frail older persons. However, there is general consensus that comorbidity, disease susceptibility and risk of developing multiple health outcomes are commonly associated with the detection of abnormal circulating levels of several biomarkers and changes in body composition. Thus, composite measures of mobility, body composition, strength, circulating hormones and biomarkers of inflammation may help clinicians understand the severity of health status deterioration in their patients over and beyond the information provided by the simple diagnosis of diseases. Aggregate measures of these outcomes should be developed in future studies and are likely to replace the current criteria for the definition of frailty, both in research projects and in clinical practice.

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