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Aging and Disease

Part II

Guest Editors

José A. Ávila-Funes

Sara Aguilar-Navarro

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Cover figure: Senile amyloid plaques in the temporal cortex from a patient with Alzheimer's disease. Staining with Thioflavin S and acquired in a Olympus BX41 fluorescence microscope (400x). Courtesy of Braulio Martínez-Benítez, MD, Department of Pathology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico.

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Preface

One of the great achievements of the twentieth century was a dramatic increase in life expectancy. In fact, during the period of 1900/1902 to 2011, life expectancy increased from 48 to 76 years in white males and from 51 to 81 years for white females in developed countries such as the USA, whereas the total deaths per 1,000 population decreased from 17.2 to 8.0 during the same period (http://www.infoplease.com/ipa/A0005140.html). Although this may tell us that we are on the road to immortality, the resulting economic impact on society of this rise in life expectancy can not be underestimated, not only for the increased government spending in the time of pension, but also for the alarming rise in cost and, consequently, increased spending on health care.

In Part II of the thematic issues on "Aging and Disease" of this journal, several important aspects on this topic are extensively reviewed and discussed by experts in the field, including the relationship of Alzheimer's disease with abnormalities in glucose metabolism, the problem of frailty and sarcopenia in the elderly, which the author of the review rightly called "new geriatric giants", the important topics of aging and kidney transplantation and pulmonary fibrosis, and the diseases associated with immunosenescence. The issue closes with an original article presenting the results of a secondary longitudinal analysis of the Costa Rican Longevity and Healthy Aging Study on the value of frailty indices to predict adverse outcomes in the elderly population. We hope that the information contained in the collection of articles presented in Parts I and II of these thematic issues will be useful to the readers of our journal and that the topics discussed promote research on this transcendental area.

The Editors of Revista de Investigación Clínica –*Clinical and Translational Investigation*– and the Guest Editors of these thematic issues want to express their appreciation to the authors for their efforts and time invested to achieve this important journal's task as well as for providing contributions in a timely fashion.

Alfredo Ulloa-Aguirre

Editor-in-Chief

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The Role of Insulin Resistance and Glucose Metabolism Dysregulation in the Development of Alzheimer´s Disease

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ABSTRACT

Alzheimer´s disease is a chronic neurodegenerative disorder affecting millions of people worldwide, characterized by a progressive decline in cognitive functions. Factors involved in the pathogenesis of Alzheimer´s disease include metabolic alterations such as insulin resistance and hyperglycemia, both of which are also hallmarks of type-2 diabetes mellitus. The accumulation of β-amyloid peptides in the brain of Alzheimer´s patients is responsible in part for the neurotoxicity underlying the loss of synaptic plasticity that triggers a cascade of events leading to cell death. A large number of studies revealed the key role of the hippocampus and cerebral cortex in the memory and learning deficits of Alzheimer´s disease. Although ample evidence suggests a link between altered insulin action, the dysregulation of glucose metabolism, and β-amyloid accumulation in animal models and humans with Alzheimer´s, no supporting evidence was available. In this article, we review the potential toxic effects of β-amyloid in the hypothalamus, a brain center involved in the control of insulin action and glucose metabolism. Furthermore, we discuss our recent studies unraveling a novel neurotoxic action of β-amyloid that perturbs hypothalamic glucoregulation, leading to increased hepatic glucose production and hyperglycemia. These findings provide evidence for a link between β-amyloid toxicity and altered glucose metabolism. (REV INVES CLIN. 2016;68:53-8)

Key words: Alzheimer´s. Diabetes. Amyloid peptide. Glucose metabolism. Insulin resistance. Hypothalamus. Liver.

THE ROLE OF β-AMYLOID PEPTIDES IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in elderly people. It is characterized by a progressive loss of cognitive abilities including a pronounced memory deficit. Neuropathological analysis of AD brains shows extensive cortical atrophy caused by severe neuronal loss. At the histological level, hallmarks of the disease are the presence of neurofibrillary tangles and neuritic plaques surrounded by extensive areas of inflammation (astrogliosis and microglial activation), mainly the cerebral cortex and hippocampus[1]. The

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principal constituent of the neurofibrillary tangles is the cytoskeletal protein tau in its hyperphosphorylated form. Neuritic plaques instead, consist of abnormal depositions of β-amyloid (Aβ) peptides of varying length, predominantly the 40 and 42 amino acid residue peptides known as $A\beta_{1-40}$ and $A\beta_{1-42}$, respectively, surrounded by dystrophic neurites. Aβ peptides are produced after proteolytic cleavage of the Aβ precursor protein (AβPP) through the action of the β- and γ-secretases in the so-called amyloidogenic pathway. In contrast, in the non-amyloidogenic pathway the action of α- and γ-secretases on AβPP prevents the production of Aβ peptides[2-4] (Fig. 1).

Extensive evidence has demonstrated the neurotoxic effect of Aβ peptides on synaptic transmission and neuronal plasticity in experimental models *in vitro* and *in vivo*. For example, overproduction of Aβ in dendrites and axons not only reduces the number of synapses but also their plasticity[5,6]. Transgenic mouse models of AD revealed a marked decrease in the density of dendritic spines as well as severe disturbances in neurotransmission, resulting in altered neuronal plasticity[7,8]. These alterations in dendritic spines clinically correlate with symptoms in AD patients[9]. The neurotoxic effects of Aβ peptides are more severe and harmful when they are caused by Aβ soluble oligomers since these block long-term synaptic potentiation, producing severe damage of synaptic plasticity with the consequent negative impact on memory and learning[10,11]. The accumulation of abnormal fibrillary deposits of Aβ or their soluble forms leads to permanent synaptic alterations in the brain thus impacting AD progress.

In vivo rodent experimental models have been widely used to understand the neurotoxic mechanisms of Aβ peptides and their key role in the neurodegenerative damage leading to dementia in AD[12,13]. Studies of chronic infusions of Aβ into the cerebral ventricles of rats showed a pattern of extensive neuronal degeneration and death[14], alterations in hippocampal synaptic transmission and plasticity[15], and a deficit in the levels of neurotransmitters, including acetylcholine, dopamine, and certain neuropeptides[16,17]. These changes are similar to those observed in AD patients and are highly associated with behavioral alterations. Studies on the effects of co-infusing Aβ in the hippocampus of rodents have demonstrated a severe behavioral deficit of spatial memory[18-20]. These pharmacological interventions have been useful in the study of the effects of Aβ peptides on memory and learning, similar to those observed in AD patients.

THE HYPOTHALAMUS AND ALZHEIMER'S DISEASE

The neuropathology of AD affects cortical as well as subcortical structures of the central nervous system (CNS). Neuritic plaques observed in AD patients are distributed predominantly in the hippocampus and neocortex where dense deposits of Aβ peptides are observed[21]. Morphological studies using immunohistochemical methods have identified a kind of neuritic plaque called diffuse or amorphous plaque in the hypothalamus of patients with AD. Diffuse plaques are formed by deposits of fine fibers of Aβ, where dystrophic dendrites around the plaques and an Aβ dense center are not present as observed in the classical form of neuritic plaques[22]. However, the presence of neuritic plaques with a much lower density than those found in the hippocampus or entorhinal cortex have been observed in the hypothalamus of humans affected with AD[23]. In the hypothalamus, the distribution of diffuse plaques is wider than that of neurofibrillary tangles. Postmortem studies in the brains of AD patients have shown that diffuse plaques are distributed from the caudal region of the pre-optical area to the pre-mammillary region of the hypothalamus, including the medial and lateral region. This is in contrast with the distribution of neurofibrillary tangles whose distribution is restricted to the lateral and posterior region of the hypothalamus[24,25]. Only the region of the supraoptic nucleus and the magnocellular part of the paraventricular nucleus of the hypothalamus appears to be free of diffuse plaques and neurofibrillary tangles[25].

THE ROLE OF THE MEDIOBASAL HYPOTHALAMUS IN THE REGULATION OF INSULIN ACTION

The regulation of insulin action and glucose metabolism is an extremely complex function that requires the synchronized communication between various systems in order to integrate biochemical, hormonal, and neurogenic signals arising in peripheral tissues and organs such as the liver, skeletal muscle, or adipose tissue. These signals are relayed to the brain

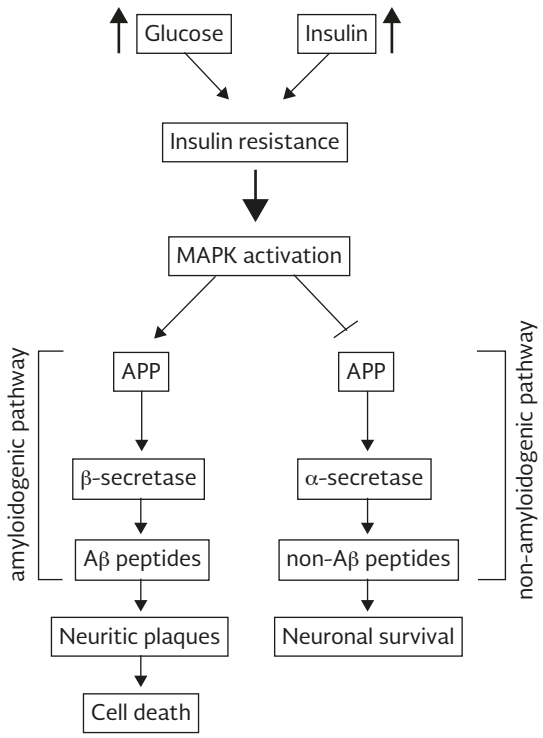
areas responsible for the control of food intake, body weight, and energy metabolism[26,27]. Circulating nutrients are derived from two main sources: (i) exogenous, produced by the digestion of ingested food, and (ii) endogenous, carbohydrates and lipids produced by the liver. Circulating nutrients, such as glucose, lipids and amino acids, increase the plasma levels of leptin and insulin. These hormones activate efferent pathways in the hypothalamus, which then send signals to inhibit food intake and the production of glucose by the liver[28,29]. Insulin or leptin administration in the hypothalamus induces rapid changes in glucose mobilization from peripheral tissues, mainly the liver and skeletal muscle[30,31]. The ability of systemic insulin to suppress hepatic glucose production is due in part to the activation of insulin receptors located specifically in the arcuate nucleus of the mediobasal hypothalamus (MBH)[31].

The MBH has been proposed as a key neuronal center in the control of glucose metabolism. Perturbations of the homeostatic hypothalamic circuits are sufficient to produce obesity and insulin resistance[29]. Activation of insulin signaling in the MBH, including the insulin receptor and phosphoinositide-3-kinase (PI3K) as well as the activation of ATP-dependent potassium (K_{ATP}) channels constitutes a strong direct stimulus to trigger neurogenic signals to the liver, via the efferent vagus nerve, to suppress hepatic glucose production[32]. Other studies in this direction implicate the interleukin-6/signal transducer and activator of transcription 3 (IL-6/STAT3) signaling pathway in the inhibition of gluconeogenic enzyme transcription in the liver[33]. All these studies revealed a key role of the MBH in the fine-tuning of insulin action for the control of glucose metabolism.

RELEVANCE OF INSULIN IN ALZHEIMER'S DISEASE

The presence of insulin and the insulin receptor (IR) in the CNS suggests that the brain is a target for the action of insulin. Indeed, insulin exerts multiple effects in the brain, including neurotrophic, neuromodulatory, and neuroendocrine actions. Insulin reaches the brain via the blood brain barrier (BBB) or, in some instances, through its local production in the brain[34]. IR has been found in high concentrations in several areas of the brain, including the olfactory bulb, hypothalamus, hippocampus, and cortex of rodent and human brains[35]. Some of the effects of IR activation on food intake regulation, energy metabolism, and reproductive function have been studied in genetically modified mice models that do not express the IR in the brain. These animals display a phenotype characterized by increased food intake; diet-induced obesity; increased body adiposity; elevated circulating insulin, leptin, and triglycerides; mild insulin resistance; and altered reproductive function[36,37]. On the other hand, abundant evidence suggests a role for IR in the modulation of synaptic activity in the CNS through its effects on the release and re-uptake of neurotransmitters[38-40]. The presence of functional IRs in the hippocampus and cerebral cortex are important for cognitive function[41,42]. To demonstrate the effects of insulin on cognition, rats were treated with streptozotocin (a diabetogenic toxin) in the third cerebral ventricle, causing a deficit in energy metabolism, memory, and learning[43]. The molecular mechanisms by which insulin affects cognitive functions have been examined in studies of insulin signaling in AD. For instance, it has been reported that IR expression is increased, while its tyrosine kinase activity is decreased, in the brain of patients with AD, suggesting defects of insulin signaling[44]. More recent studies have indicated that insulin regulates the metabolism of the proteins Aβ and tau, the main components of senile plaques and neurofibrillary tangles, respectively, and constituents of the characteristic neuropathological lesions in AD. The association of tau with the microtubules is regulated through protein phosphorylation by protein kinases including glycogen synthase kinase 3β (GSK-3β). The GSK-3β is a main component of the insulin signaling pathway, and its activity is controlled by the binding of insulin and insulin-like growth factor 1 (IGF-1) to the IR[45]. Studies in neuronal cultures demonstrated that insulin and IGF-1 decreases tau phosphorylation and promotes its binding to microtubules via GSK-3β inhibition by PI3K[46]. It has also been reported that insulin and IGF-1 transiently increase tau phosphorylation on specific residues as a consequence of GSK-3β activation by Fyn tyrosine kinase[47]. In the case of interaction of Aβ peptides with insulin signaling, some studies have proposed that insulin may promote the accumulation of Aβ peptides through the stimulation of AβPP processing after activation of the mitogen-activated protein kinase (MAPK) pathway[48] (Fig. 1). Other studies have proposed a role for the insulin-degrading enzyme (IDE) in the molecular cascade leading to the abnormal accumulation of Aβ in the brain of people with AD. IDE is a metalloproteinase that degrades insulin and other small peptides, including Aβ.

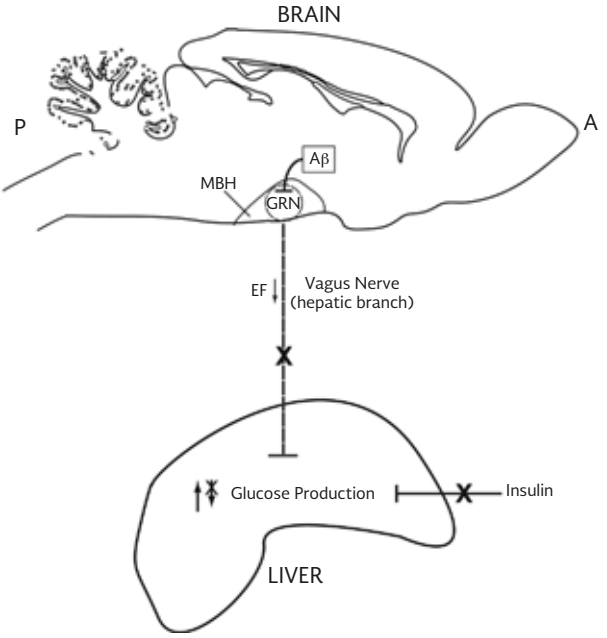
Figure 1. Schematic representation of the role of insulin signaling in the metabolism of β-amyloid and the impact of hyperglycemia and hyperinsulinemia in the activity of the amyloidogenic pathway. High insulin and glucose levels are proposed to promote the abnormal accumulation of toxic β-amyloid peptides as a result of the stimulation of β-amyloid precursor protein processing mediated by insulin-dependent activation of the mitogen-activated protein kinase pathway. Conversely, high insulin and glucose block the activation of the non-amyloidogenic pathway, decreasing the production of non-toxic soluble peptides that promote neuronal survival. MAPK: mitogen-activated protein kinase; Aβ: β-amyloid; APP: Aβ precursor protein.



It has been hypothesized that the binding of insulin to IDE not only stimulates the degradation and disposal of Aβ during hyperinsulinemia, but also promotes the formation of Aβ plaques[49].

Recently, we have shown that an Aβ short fragment, the amino acid sequence 25-35 ($A\beta_{25-35}$) participates in the dysregulation of the glucose metabolism in a non-diabetic animal model. We tested the hypothesis that $A\beta_{25-35}$ may have a toxic action in the MBH that perturbs central glucoregulation. To this aim, we first determined whether short-term exposure of the hypothalamus to $A\beta_{25-35}$ alters the circulating levels of both glucose and insulin by infusing $A\beta_{25-35}$ in the MBH of young rats. We observed that the acute intrahypothalamic infusion of $A\beta_{25-35}$ increased plasma glucose

Figure 2. Schematic representation of the brain-liver interaction proposed to mediate the central toxic effect of $A\beta_{25-35}$ on liver glucose metabolism. Acute exposure of the mediobasal hypothalamus to $A\beta_{25-35}$ (shown in this rat sagittal brain middle section) blocks the glucoregulatory neuronal activity that normally sends appropriate neurogenic inhibitory signals through the hepatic vagal efferent innervation. The net result is an increase of liver endogenous glucose production and hyperglycemia. Aβ: $A\beta_{25-35}$ peptide; MBH: mediobasal hypothalamus; GRN: glucoregulatory neurons; EF: efferent flow; A: brain anterior face; P: brain posterior face.



and insulin levels in comparison with the control peptide animal group. To gain insight into the mechanisms by which $A\beta_{25-35}$ increased glucose levels, we examined its effect during the course of pancreatic basal insulin clamps designed to maintain fixed and basal circulating insulin levels. Glucose kinetics measurements performed during the clamps showed that $A\beta_{25-35}$ caused a marked increase of endogenous glucose production, reflected by the decrease in the glucose infusion rate to maintain normal glucose levels and by the failure of the system to efficiently suppress glucose production by the liver. Furthermore, we did not observe changes in glucose utilization by peripheral tissues in the animals infused with $A\beta_{25-35}$ in the MBH[50]. These results unraveled a novel neurotoxic action of Aβ that perturbs hypothalamic glucoregulation, leading to increased hepatic glucose production and hyperglycemia. Furthermore, our findings provide a previously lacking piece of experimental evidence for a direct link between Aβ toxicity and altered glucose metabolism (Fig. 2).

DIABETES AND ALZHEIMER'S DISEASE

Type 2 diabetes mellitus (DM2) is one of the most common metabolic disorders and its prevalence and incidence has increased worldwide. Several epidemiological studies have suggested that DM2 is involved in the development of dementia in AD[51-53]; however, the factors linking DM2 with AD are largely unclear. Some of the risk factors proposed to play a role in the neurodegenerative process and the progression of dementia in AD include: hyperglycemia, insulin resistance, oxidative stress, activation of inflammatory cytokines, and damage to the micro/macrovascular system[54]. Clinical studies of patients with AD have yielded intriguing results. For example, high fasting blood insulin accompanied by low insulin levels in the cerebrospinal fluid (CSF) has been reported in patients with AD[55]. In contrast, studies of AD patients with varying degrees of severity of the disease display different profiles since individuals with milder forms of AD display high fasting plasma glucose and insulin, while in individuals with severe AD the hyperglycemia is not accompanied by hyperinsulinemia[56]. Prospective studies of patients with milder forms of AD and dementia progression revealed that low circulating insulin was associated with a decrease of memory facilitation, suggesting a cause-effect relationship between levels of plasma insulin and cognitive function. In this respect, short-term follow-up studies have demonstrated that hyperinsulinemia improves memory as long as circulating glucose is maintained at fasting levels. Conversely, hyperglycemia does not affect cognitive function as long as circulating glucose remains within normal basal limits[57,58]. The study of individuals with a diagnosis of AD that showed alterations of circulating insulin and dementia progression suggested that insulin was involved in the regulation of cognitive function. Consequently, it is clear that insulin may play a relevant role in the pathophysiology of AD.

CONCLUSIONS

In conclusion, studies on the molecular and cellular mechanisms involved in the pathophysiology of AD and its relationship with insulin and glucose metabolism support the idea that the metabolic alterations of DM2 are strongly associated to the development of AD. Importantly, our recent studies in rodents provided a piece of evidence supporting the novel concept that Aβ toxic action in the hypothalamus causes a dysregulation of glucose metabolism directly linking altered insulin action with AD. Further studies are required to better understand the details of this relationship and its causal role, if any, in the onset and progression of AD.

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Frailty and Sarcopenia: The New Geriatric Giants

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ABSTRACT

In the last decade, it has become clear that older persons who are frail or sarcopenic have very high rates of functional deterioration, hospitalization, and death. Recently, it has become recognized that simple screening questionnaires, e.g., the FRAIL and SARC-F, perform as well as more complex testing for the physical phenotype screen and sarcopenia. In this article, we provide a simple algorithm for the management of frailty. The multiple factors responsible for the pathogenesis of sarcopenia are reviewed, focusing on the importance of age-associated loss of motor units innervating muscle. Management of sarcopenia includes resistance exercise, leucine-enriched protein, and vitamin D. A number of newer drugs are under development. General practitioners should be encouraged to screen for frailty and sarcopenia in older persons. (REV INVES CLIN. 2016;68:59-67)

Key words: Frailty. Sarcopenia. Geriatrics. Screening tools. Aging.

In 1976 Bernard Isaacs documented the giants of geriatrics as: impaired vision and hearing, instability and falls, incontinence (fecal and urinary), and intellectual impairment (dementia and delirium)[1,2]. He considered these the conditions that were present in frail older persons. Frailty can be considered to be a state of vulnerability that increases the chance of an older person having functional deterioration, hospitalization, or death[3]. In 2001 Fried, et al.[4] suggested that a physical phenotype (weakness [grip strength], slowness [walking speed], low level of physical activity, self-reported exhaustion, and unintentional weight loss > 4.5 kg in one year) (Table 1) would be useful to recognize frail individuals. It is particularly useful for recognizing persons at high risk of developing functional impairment (loss of activities of daily living)[5]. This approach has become enormously successful for research purposes, but has not been included in general geriatric practice[6-9].

Recently, a simple five question FRAIL scale has been developed which is easily utilized in the clinical setting (Table 2)[10-12].

The term "sarcopenia" was introduced into the literature by Irv Rosenberg in 1995[13]. It was defined as an abnormal loss of muscle associated with aging and it has been validated to predict functional decline[14]. However, Manini and Clark[15] pointed out that it was muscle power and not muscle mass that

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Table 1. Fried's frailty phenotype[4]

A. Characteristics of frailty
   Shrinking: Weight loss (unintentional)
      Sarcopenia (loss of muscle mass)
   Weakness
   Poor endurance (exhaustion)
   Slowness
   Low activity

B. Cardiovascular Health Study Measure
   Baseline: > 4.5 kg lost unintentionally in prior year
   Grip strength: lowest 20% (by gender, body mass index)
   "Exhaustion" (self-report)
   Walking time/15 feet: slowest 20% (by gender, height)
   Kcals/week: Lowest 20% (males < 383 Kcals/week; females: < 270 Kcals/week)

C. Presence of Frailty
   Positive for frailty phenotype: ≥ 3 criteria present
   Intermediate or pre-frail: 1 or 2 criteria present

Table 2. The FRAIL Scale: A rapid, validated scale for the detection of frailty

3 or more positive answers – frail
1 or 2 positive answers – pre-frail

F atigue (have felt tired most or all of the time in past 4 weeks)
R esistance (have difficulty or unable to climb a flight of stairs)
A erobic (have difficulty or unable to walk a block)
I llness (have more than 5 illnesses)
L oss of weight (have lost more than 5% of weight in past 6 months)

Adapted with permission from Morley, et al.[10].

was the predominant feature that led to loss of functional status. Thus, in 2010 the European Consensus on the Definition and Diagnosis of Sarcopenia changed the definition of sarcopenia to be "muscle loss together with a loss of function as measured by either walking speed or grip strength[16]. Subsequently, four other similar definitions of sarcopenia were published with somewhat different cutoff points[17-20]. In view of the finding that the six FRAX questions without measuring bone mineral density were predictive of fracture risk, we developed a five-question scale (SARC-F) to detect muscle dysfunction in older persons[21-23] (Table 3).

We believe that frailty and sarcopenia should now be recognized as the new geriatric giants. The availability of rapid scales allows primary care physicians to recognize these conditions and to either treat them or refer persons with these syndromes to a geriatrician[24].

FRAILTY PHENOTYPE

There is now international consensus that frailty is a measurable clinical syndrome that recognizes persons at increased vulnerability to stress who may have treatable conditions[10]. It is felt that all persons over 70 years of age should be screened for frailty. Utilizing the physical phenotype of Fried (Cardiovascular Health Study) or FRAIL, persons are considered frail if they have three or more criteria, and pre-frail if they have one or two components. Frailty overlaps with disability, but not all

Tabla 3. SARC-F screen for sarcopenia

Component	Question	Scoring
Strength	How much difficulty do you have in lifting and carrying 10 pounds?	None = 0 Some = 1 A lot or unable = 2
Assistance in walking	How much difficulty do you have walking across a room?	None = 0 Some = 1 A lot, use aids, or unable = 2
Rise from a chair	How much difficulty do you have transferring from a chair or bed?	None = 0 Some = 1 A lot or unable without help = 2
Climb stairs	How much difficulty do you have climbing a flight of ten stairs?	None = 0 Some = 1 A lot or unable = 2
Falls	How many times have you fallen in the last year?	None = 0 1-3 falls = 1 4 or more falls = 2

Adapted with permission from Malmstrom, et al.[21].

disabled persons are frail and about 70% of the frail are not disabled[25]. Sarcopenia is one of the causes of frailty, but similarly, not all frail persons are sarcopenic and not all sarcopenic persons are frail[26].

There are numerous other frailty scales that have been developed. Some of these are similar to the physical phenotype of frailty (e.g., Study of Osteoporotic Fractures[27], the Frailty Instrument for Primary Care[28], or the Survey of Health Ageing and Retirement in Europe, SHARE-FI)[29]. Others are broader and include psychosocial factors, e.g., the Groningen and Tilburg Frailty Indices[30-33].

The prevalence of physical frailty increases with aging from under 5% in community dwelling persons aged 65-75 years to about 25% in persons who are 85 years of age or older34. Table 4 provides examples of the prevalence of physical frailty in community dwelling persons in different countries[35-55].

A separate approach to frailty has been developed by Rockwood, et al.[56] utilizing the Canadian Health Survey. This has been termed the Frailty Index (FI). This is developed by summing the number of diseases and physical and psychosocial deficits present in an older person. Scales vary from 30 to 100 items. While the FI is highly predictive of poor outcomes, it is much more a comorbidity or multimorbidity index than a true frailty measure. It fails to separate frailty from the underlying comorbidities that may be its cause and it includes disabilities that it is supposed to predict. As such, one can question whether or not it is a true frailty index, though it clearly has a utility as a predictive index.

MANAGEMENT OF FRAILTY

The FRAIL index can be used as a guideline for management. It has been successfully used in the community to recognize frail persons[57]. Persons who answer that they are fatigued should be screened for depression using either the Patient Health Questionnaire 9 (PHQ-9) or the Geriatric Depression Scale[58]. Sleep apnea is a common cause of fatigue and should be excluded by querying whether or not the person stops breathing at night, snores excessively, or falls asleep while driving or in the middle of a conversation. Hypothyroidism, vitamin B_{12} deficiency, and anemia are common causes of tiredness. Low blood pressure, especially orthostatic hypotension or postprandial hypotension, also cause fatigue[59,60].

Problems with the questions about resistance and aerobic activity suggest sarcopenia. This can be treated with resistance exercise, 1,000 IU vitamin D and a leucine-enriched essential amino acid supplement (see section on sarcopenia treatment).

Persons who have multiple illnesses usually have polypharmacy, which often leads to drug side effects[61-63]. Anticholinergic drugs are particularly likely to lead to central nervous system side effects and fatigue[64,65]. Reduction of polypharmacy often can reduce drug side effects and improve quality of life[66,67].

Weight loss in older persons has a variety of treatable diseases, which can be recognized using the MEALS-ON-WHEELS mnemonic[68-70]. For persons with chronic obstructive pulmonary disease, multiple small meals may overcome the dyspnea associated with the thermic effect of eating[71]. Further caloric supplementation can slow the progression of weight loss[72-74].

Figure 1 provides a simple algorithm for the treatment of frailty.

SARCOPENIA

Sarcopenia is an inevitable consequence of aging as demonstrated by the decline in the women's world record for the long jump, which is 7.44 m for the young and 1.72 m for ninety year olds. Aging results in muscle fiber size heterogeneity with a predominant loss of Type II muscle fibers and a decline in satellite cells[75]. Sarcopenia needs to be differentiated from cachexia, which is due predominantly to an increase in proinflammatory cytokines due to diseases[76,77]. Fiber size variability is not present in cachexia.

A major component of muscle loss with aging is due to a loss of motor units innervating muscle[78]. Over the lifespan there is a loss of approximately 25% of motor neurons innervating type II muscle fibers[79]. Damage to motor units can be detected by measuring circulating C-terminal agrin[80]. The accelerated loss of muscle mass that occurs in persons with diabetes mellitus is due to the decreased muscle innervation coupled with decreased blood flow to muscle[81-83].

Tabla 4. Prevalence of frailty in older persons in different countries

Country	Age (years)	Percentage (%)	Notes
USA, CHS[7]	65-74 85+	3.9 25.0	
AAH[36]	49-65 58-74	2.7 (7.5) 8.6	ADL dependence included in parenthesis
WHAS[35]	70-79	11.3	Women only
Canada GLOW[38]	55+	15.0	Women only
Mexico SADEM[39]	60+	15.7	
Brazil FIBRA-RJ[40]	65+	9.1	
Peru[41]	60+	27.8	
Columbia[42]	60+	12.2	Rural, living in Andes
Europe[37]	65+	17.0	5.8% in Switzerland 27.3% in Spain
United Kingdom[43]	65+	8.1	
Ireland[44]	65+	6.0	
Turkey[45,53]	65+	27.8 (10)	FRAIL in parenthesis
China[46]	60+	15.1	Only included diabetics
Japan[47]	65+	9.3	
Hong Kong[11]	65+	14.0	
Taiwan[48]	65+	4.9	
Korea[49]	65+	7.8	
Malaysia[50]	60+	5.7	
Singapore[51]	55+	2.5	
Australia, Western[54,55]	70+ 70+	20.6 5.6	Men only using FRAIL scale Women only
Australia Longitudinal Study on Women's Health[52]	85+	16.2	Women only

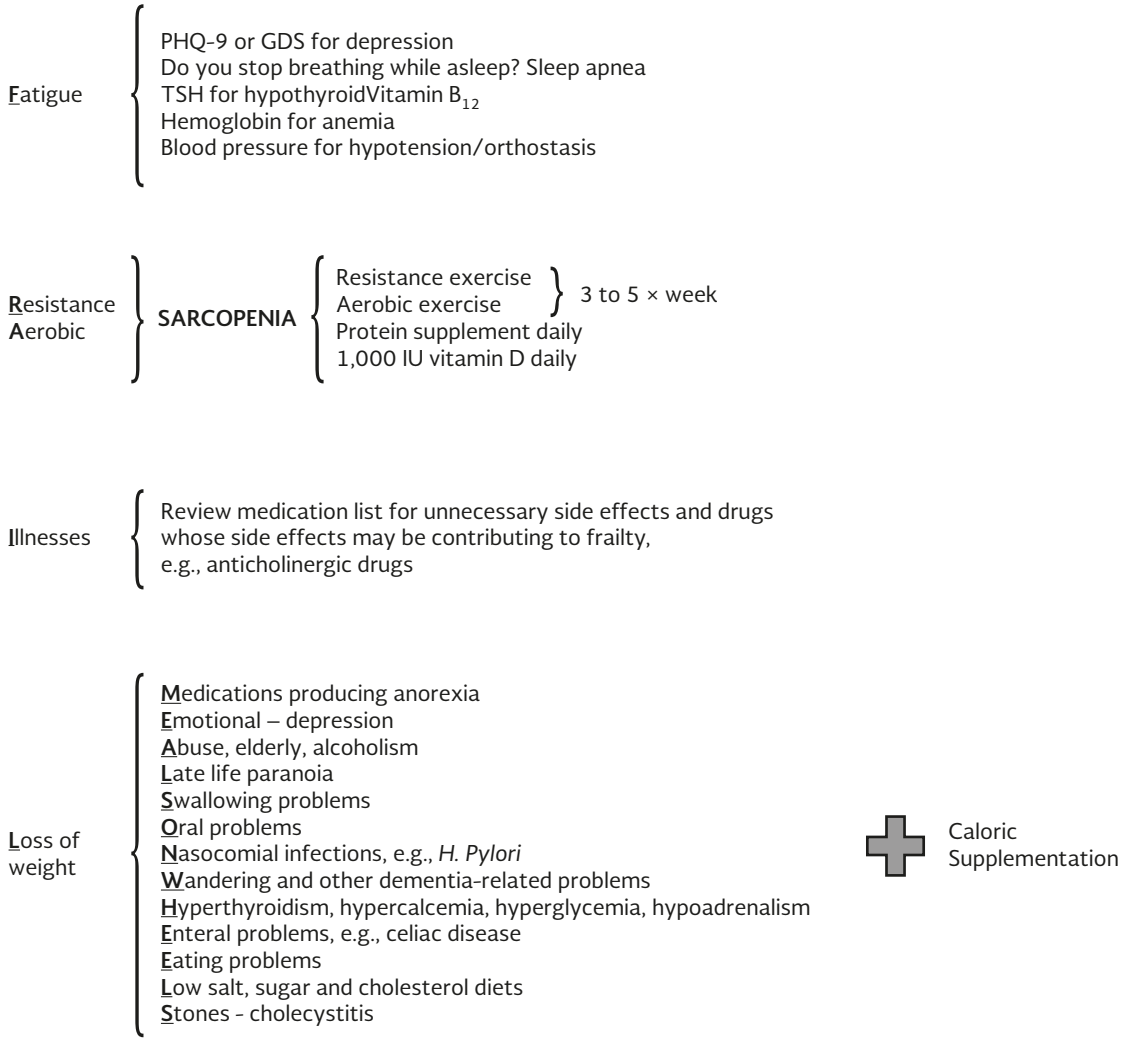
AAH: Action Against Hunger; ADL: activities of daily living; SADEM: Study on Aging and Dementia: CHS: Cardiovascular Health Study; WHAS: Women's Health and Aging Studies.

The development of sarcopenia with aging appears to be related to a variety of age-related factors. Anabolic hormones, especially testosterone, show a decline of about 1% per year from the age of 30 years[84]. This decline is closely related to both the loss of muscle and strength that occurs with aging[85]. The decline in growth hormone leads to a decline in insulin-like growth factor-1 and mechano growth factor[86]. This decline is related to the loss of muscle mass, but not necessarily muscle power[87]. With aging, there is an anorexia of aging which leads to muscle loss[88]. There is also a decline in activity with aging, further causing muscle to be less functional[89]. Levels of 25(OH) vitamin D decline longitudinally with aging[90]. This is both due to a decrease in the ability of cholecalciferol in the skin to make 25(OH) vitamin D and a decline in vitamin D absorption, as well as a decrease in sun exposure and the use of sunblock[91]. Decreased blood supply to the muscles due to atherosclerosis leads to muscle hypoxia. Insulin resistance associated with aging results in increased fat infiltration into muscle, leading to a decline in muscle function[92]. Parabiosis experiments between young and old mice have found a role of the circulating factor –growth differentiation factor-1– in age-related muscle loss[93]; Low-grade proinflammatory cytokine production that occurs with aging results in loss of muscle mass and function[94]. Finally, mitochondrial dysfunction that is associated with aging leads not only to oxidative damage of muscle, but also a reduction in the ability to generate energy to allow muscles to function properly[95]. The factors involved in the pathophysiology of sarcopenia are outlined in table 5.

MANAGEMENT OF SARCOPENIA

Since the original manuscript by Fiatarone, et al.[96] demonstrating that resistance exercises can improve strength in 90-year-old nursing home residents, numerous papers have been published supporting the utility of

Figure 1. Algorithm for management of frailty.



resistance exercise in improving muscle strength in persons with sarcopenia[97]. The LIFE study found that aerobic exercise slowed lower limb functional decline[98]. Singh, et al.[99] showed that resistance exercise twice a week for a year markedly improved clinical outcomes in older persons following hip fracture[100].

There is evidence that older persons who have lost muscle require 1.0-1.2 g/kg/day of protein to restore the loss of muscle mass[100,101]. This protein should be leucine-enriched essential amino acid based[102]. A number of studies have suggested that the addition of protein to exercise can further increase muscle performance[103-106]. The PROVIDE study showed that protein supplementation together with vitamin D increased muscle mass and the ability to do chair stands in persons with sarcopenia[107].

Replacement of vitamin D in vitamin D-deficient persons increases muscle strength and prevents falls[108,109]. Vitamin D supplementation does not increase muscle mass.

Testosterone can increase muscle mass in persons with low testosterone[110,111]. Higher doses of testosterone are required to improve muscle strength and/or power[112-114]. Testosterone improves function in older persons with frailty[115-117]. Testosterone activates beta-catenin to increase muscle mass and at high doses recruits satellite cells to enhance muscle strength[118]. In general, testosterone has minimal side effects[119,120]. However, there is some evidence that within the first year of treatment, testosterone increases cardiovascular disease[121]. This may be due to excessive increase in hematocrit where this is poorly monitored or due

Table 5. Factors involved in the pathophysiology of sarcopenia

Factor	Effect	
	Loss of muscle mass	Loss of muscle strength
1. Decreased physical activity	Yes	Yes
2. Decreased food (protein) intake	Yes	No
3. Decreased testosterone	Yes	Yes
4. Decreased growth hormone and Insulin Growth Factor I	Yes	No
5. Decreased DHEA	Small	No evidence
6. Vitamin D deficiency	No	Yes
7. Insulin resistance	No	Yes
8. Decreased growth differentiation factor-1	Yes	No evidence
9. Atherosclerosis	Yes	Yes
10. Decreased motor units	Small	Yes
11. Proinflammatory cytokine excess	Yes	Yes
12. Mitochondrial dysfunction	No	Yes

DHEA: dehydroepiandrosterone.

to increased relaxation of the coronary arteries resulting in rupture of unstable plaques[122]. A number of selective androgen receptor modulators have been developed. Unfortunately, to date they have not been demonstrated to be more effective than testosterone and there is insufficient evidence to determine if they have a better safety profile[123,124].

Growth hormone increased muscle mass and nitrogen retention[125,126]. It does not improve muscle strength and thus should not be used to treat sarcopenia.

Ghrelin is a hormone that is produced from the fundus of the stomach and enhances growth hormone release, food intake, and memory[127]. Anamorelin, a ghrelin agonist, has been shown to increase food intake and muscle mass, but not muscle function, in persons with cancer[128].

Antibodies to myostatin and the activin II receptor have been developed. Myostatin antibodies increase muscle mass and muscle fiber diameter in mice[129]. There is some evidence that they may have similar effects in humans with sarcopenia[130]. Activin II receptor ligand traps have powerful effects on increasing muscle and bone mass, but side effects have led to their development being halted[131]. Novartis has developed a direct antibody to the activin II receptor that has shown positive effects in persons with inclusion body myositis[132].

Other drugs under development to treat sarcopenia include the angiotensin converting enzyme inhibitor (perindopril), fast skeletal troponin activators (tirasemtiv) and mixed beta agonist/antagonist (espindolol)[133-135].

OSTEOSARCOPENIA

There is increasing evidence that osteoporosis and sarcopenia frequently coexist[136]. Men with sarcopenia have an increased risk of hip fracture[137]. Exercise increases muscle strength and muscle contraction directly enhances bone mineral density and bone quality[138]. In addition, vitamin D has direct effects on bone and muscle[139]. However, recent studies have suggested that the interaction between muscle and bone is due to an extremely complex bidirectional communication between both muscle and bone chemokines[140]. Further, both adipose tissue and cartilage also produce paracrine substances that modify the function of muscle and bone. Among the myokines that modulate bone are proinflammatory cytokines, myostatin, fibroblast growth factor-2, insulin-like growth factor-1, Tmem119, and osteoglycin[141]. Bone chemokines include the osteocyte-derived prostaglandin E2 and WnT3a as well as osteoblastic products such as osteocalcin and sclerostin. Under-carboxylated osteocalcin also plays a hormonal role by increasing the function of insulin receptors to increase glucose entry into cells.

COGNITIVE FRAILTY

Cognitive frailty has been defined as physical frailty coupled with cognitive impairment (CDR 0.5)[142]. The concept was first recognized in older Mexican Americans in 2008[143] and in 2011 was also described in the Mexican study of Nutritional and Psychosocial Markers of Frailty[144]. Persons with the combination of cognitive impairment and the physical frailty phenotype are more likely to develop disability, a decline in instrumental activities of daily living, and hospitalization[145]. The coexistence of physical frailty and cognitive impairment is not surprising given the fact that proinflammatory

cytokines play a role in the pathophysiology of both conditions[146], and white matter hyperintensity is related to both cognitive impairment, decline in walking speed, and falls[147]. The IAGG consensus conference on "Brain Health" has stated that there is a need for further research into this important relationship[148].

CONCLUSIONS

Over the last decade, frailty and sarcopenia have risen to become the true modern giants of geriatrics. While frailty and sarcopenia overlap, about a third of persons with sarcopenia do not have frailty, and similarly, all frail persons do not have sarcopenia[149,150]. The recent development of rapid, simple screening tests for both conditions (FRAIL and SARC-F) has made it easy for clinicians to identify them. In this article we provide a simple algorithm to treat both physical frailty and sarcopenia. It is hoped that this approach to secondary prevention will lead to a reduction in disability in older persons.

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Aging Kidney Transplantation

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ABSTRACT

There are several immunological and non-immunological factors related to renal graft deterioration, and histological lesions such as interstitial fibrosis and tubular atrophy overlap with those observed in aging kidneys. Consequently, it has been proposed that kidney transplant senescence could contribute to graft loss. The process of cell senescence displays characteristics such as an increased expression of specific aging suppressor genes, shortened telomeres, mitochondrial changes, increased expression of negative regulators of the cell cycle, and immunological senescence. Additionally, tubular frailty characterizes the aged kidney, making it more susceptible to ischemia, reperfusion, toxic injury, and consequently, to inflammation. Moreover, renal tissue injury predisposes the older graft not only to progressive deterioration due to glomerular hyperfiltration, but also triggers acute rejection due to increased immunogenicity. In conclusion, renal graft senescence is a complex process, and its better understanding will help the nephrologist in its management in order to achieve a longer graft survival. (REV INVES CLIN. 2016;68:68-74)

Key words: Aging. Senescence. Kidney. Transplant.

INTRODUCTION

Kidney transplant is the therapy of choice for patients with end-stage chronic renal disease, increasing their quality of life, survival, and longevity[1].

Organ shortage continues to be a major issue in kidney transplantation, and counteracting this problem is the current acceptance of older donors[2]. However, long-term graft survival is influenced by donor age, being one mechanism how aging increases acute kidney injury, and reduces tissue regenerative capability[3-5]. Moreover, there are several immunological and non-immunological factors related to renal graft deterioration, and histological lesions such as interstitial fibrosis and tubular atrophy overlap with those observed in aging kidneys. Consequently, it has been proposed that kidney transplant senescence could contribute to graft loss[3].

The complex process of cell senescence displays characteristics such as shortened telomeres, increased expression of negative regulators of the cell cycle, increased expression of specific aging suppressor genes, and immunological senescence[6,7].

Additionally, tubular frailty is one of the major changes that characterize the aged kidney ('nephro-geriatric giants'), because old kidneys are more susceptible to

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ischemia, reperfusion, and toxic injury, and this damage contributes to a cascade of inflammation[3,5,6]. Renal tissue injury, irrespective of the cause (aging, ischemia, or toxic agents) predisposes older grafts not only to progressive mass deterioration due to glomerular hyperfiltration, but also to trigger acute rejection due to an increase in their immunogenicity. This increased immunogenicity can be explained by a rise in proinflammatory cytokines and increased expression of major histocompatibility complex antigens on epithelial and endothelial cells[8,9].

To better understand how senescence influences the survival of kidney transplants, the main graft aging mechanisms previously mentioned are explained in detail as follows[10].

KLOTHO GENE

Klotho (kl) is one of the main "aging suppressor" genes since it facilitates the removal of reactive oxygen species (ROS) [11,12]. It has been documented that Klotho protein activates the forkhead box O (FoxO) transcription factors, which facilitate ROS removal and confer oxidative stress resistance by inducing manganese superoxide dismutase expression and regulating the apoptotic process[11-13]. Conversely, a defect in its expression (Klotho anti-aging protein) leads to symptoms that resemble human senescence, including reduced lifespan, arteriosclerosis, infertility, osteoporosis, cardiac valve calcification, skin atrophy, emphysema, and osteoporosis[11].

Klotho overexpression leads to aging suppression and consequently to longer lifespan in animal models[14], while angiotensin II, which is involved in age-related organ damage in mice, plays a central role in reducing renal *Klotho* gene expression. Besides, *Klotho* gene induction could protect the kidney against angiotensin II-induced damage, and angiotensin II receptor antagonists (e.g., losartan) increase *Klotho* expression[11,15]. On the contrary, angiotensin II-induced oxidative stress can downregulate *Klotho* expression[11].

It is worth mentioning that Klotho protein mediates nitric oxide vascular production, promoting vessel relaxation and endothelial dysfunction improvement in experimental atherogenesis models[12]. Even though Klotho trans-membrane protein is mainly expressed in the choroid plexus and kidney (distal tubules), where it functions as a coreceptor for fibroblast growth factor 23, it acts on various organs suppressing the expression of multiple aging-like phenotypes[14,15]. This evidence suggests that Klotho protein, or its metabolites, can function as a humoral factor[15]. Additionally, it has also been observed that *Klotho* gene influences calcium, phosphorus, and vitamin D metabolism[13].

Klotho gene has been reported to be markedly suppressed in acute renal failure, chronic kidney disease, diabetes mellitus, as well as in acute stress states. Finally, there is also a relationship between *Klotho* gene expression and immunosuppressant drugs, which is discussed in another section of this article[12,16].

TELOMERE SHORTENING

Somatic cells have a limit in their replicative capacity (around 50 divisions), a phenomenon known as "Hayflick limit" or "replicative senescence"[3,7]. Beyond this limit, cells stop proliferating and become senescent because they are resistant to growth factor signaling, and then they arrest irreversibly in the G1 phase of the cell cycle, although they remain metabolically active, a situation that contributes to their damage, loss of mass, decrease in their physiological capacity, reduction in their resistance to stress, and finally death[3]. Telomere shortening is a heterogeneous process since it is faster in the cortex compared to the medulla in aging human kidneys, and it has been interpreted as a homeostatic mechanism to prevent neoplastic cell transformation[3].

Telomeres are located at the end of eukaryotic chromosomes, and their role is to protect them from degradation in order to maintain genome integrity and stability[17]. This protective activity of the telomeres depends on many factors such as proteins linked to their role (tumor necrosis factor receptor associated factors 1 and 2), degree of telomerase activity, and telomere length itself[17]. Telomere shortening of about 50-200 bp occurs with each cell division in a state of telomerase inactivity, and when telomere length reaches a critical value the cell starts a process of apoptosis. In this sense, individuals who inherit longer than average telomeres usually have an increased lifespan; thus, cell replicative limit has been attributed to the loss of telomeres[17,18].

In addition to from normal aging, telomere shortening has also been documented in lymphocytes from HIV patients, delayed renal graft function, acute and chronic rejection, and chronic allograft dysfunction. Telomere length was positively significantly correlated with recipient age, but negatively significantly correlated with donor age, time of dialysis before transplantation, panel reactive antibodies, and long-term creatinine concentration in graft biopsies[19]. It has been postulated that telomere erosion occurs due to graft ischemia and reperfusion. In these cases, there is a transient increase of ROS, which are DNA damage inducers, and injured tissue cannot be replaced by healthy cells, thus causing persistent inflammation and thereby scarring[3,17,18].

MITOCHONDRIAL CHANGES

Sahin et al., demonstrated that telomere attrition activates p53, which in turn binds and represses mitochondrial activity regulators (PGC-1α and PGC-1β promoters)[19]. These transcriptional changes reduce the cellular energy supply, decrease respiratory function, and increase ROS production, a potential cell senescence mechanism induced by DNA damage[19,20]. Thus, these authors found a direct link between telomere dysfunction and mitochondrial aging[19,20,23-25].

It has also been reported that PGC-1α can be stabilized in the kidneys by increasing sirtuin 1, a NAD-dependent histone deacetylase, during an anti-aging intervention such as caloric restriction[19,21,22].

Additionally, it is known that angiotensin II is implicated in the generation of both cytosolic and mitochondrial ROS[25-27]. Mitochondrial aging induces an increase in angiotensin II type 1 receptor (AT1R), as well as a decrease in type 2 receptor density, which are reversed by chronic treatment with an angiotensin II type 1 receptor blocker such as losartan[26]. Besides, AT1R genetic disruption has been shown to promote longevity and reduce age-related mitochondrial dysfunction in renal tubular epithelial cells[27].

P16, P21, AND P27 CYCLIN-DEPENDENT KINASE INHIBITOR GENES

It has been documented that renal ischemia and reperfusion is associated with overexpression of cyclin-dependent kinase inhibitor genes (CDKIG), indicating DNA damage and/or accelerated histological senescence[28].

P16^{INK4a} is a cell cycle inhibitor associated with somatic cell senescence, which is considered an indicator of premature aging secondary to stress and disease[7].

Chronically diseased native kidneys show histological changes, which are qualitatively similar but quantitatively greater than changes secondary to normal senescence. Also, they display increased P16^{INK4a} expression in glomerular and tubular-interstitial cells beyond the area affected by these structural changes, thus this extensive expression of P16^{INK4a} seems to cause these changes more than to be their consequence[10]. Regarding kidney transplantation, an increase of P16^{INK4a} has been documented in grafts with tubular atrophy, interstitial fibrosis, and impaired function (findings that affect 60% of cadaver transplants), suggesting that a part of the changes suffered by senescent grafts is induced by multiple insults associated with transplantation. Among these insults are the injuries from brain death, organ preservation, cold ischemia, transplantation process, drug toxicity, infections, hypertension, and dyslipidemia, which contribute to premature kidney aging accelerating its atrophy[7,10]. Additionally, P16^{INK4a} is induced in allografts from old donor kidneys soon after transplantation (about a week), while young non-transplanted kidneys showed very little basal expression of P16^{INK4a}, but an increased expression not until later after transplantation (about a month). Conversely, isografts have no effect on P16^{INK4a} expression[6,28,29]. Besides, it has been documented that renal cold ischemia and reperfusion are associated with up-regulated p16, p21, and p27 CDKIGs in kidney tissue, indicating DNA damage and/or accelerated histological senescence[28].

IMMUNOSENESCENCE AND INFLAMMAGING

There is a gradual deterioration of the immune system with aging, a phenomenon known as "immunosenescence", which affects both innate and adaptive (T- and B-cell) immune components[1]. However, age-related immune deficiency is more prominent in adaptive immunity, and it consists of an accumulation of anergic terminally differentiated lymphocytes, mainly due to telomeres erosion, and a deficit of fully active

naïve cells[1,30]. Regarding the innate immune system (macrophages, neutrophils, and natural killer cells), it triggers adaptive immune responses, while dendritic cells are antigen-presenting cells that function as a bridge between the innate and the adaptive immune systems[31]. Intragraft interstitial dendritic cells can increase old donor immunogenicity, but older monocyte-derived dendritic cells show an impaired phagocytosis and pinocytosis capability. Besides, aged macrophages show a significant reduction in their number as well as in their chemotaxis, phagocytosis, cytokine, and chemokine production capabilities[31]. Regarding neutrophils, they play an important role in the defense against microorganisms and in the inflammatory response. Even though there is no decrease in their number with aging, they decrease their chemotaxis and phagocytic capability[31]. Finally, natural killer activity, which plays a key role in immunity to tumoral cells and pathogens, is impaired in the elderly[4].

With regards to T-cells, thymic involution starts at the age of one year and advances fast with puberty, and it has a residual capacity of producing naïve T-cells in the elderly, but they are less functional in response than in young people[31]. Although senescence loss in thymic output does not result in significant changes in the total amount of peripheral T-cells because this is regulated via a thymus-independent expansion of mature T-cells, they have a reduced allorecognition capability[4]. This exhaustion of the immune system was documented in $CD8^+$ T-cells rather than in $CD4^+$ T-cells. This phenomenon could be attributed to the time necessary for the $CD4^+$ T-cells to become senescent, since even in extreme conditions, when $CD8^+$ T-cells shorten their telomeres relatively quickly, telomere erosion in $CD4^+$ T-cells may take years[30]. Aging characteristically increases the expression of $CD8^+$ T-cells that lack the expression of CD28. This loss of expression has been attributed to repeated antigenic stimulation and telomere erosion. Inflammation (e.g., chronic viral stimulation) or acute renal rejection increase the proportion of $CD28^-$ T-cells[3,32]. Additionally, $CD28^-$ expression has been associated to telomere shortening, replicative senescence, and proinflammatory cytokine production (interleukin 10 and interferon-γ)[3,32]. An increased proportion of $CD8^+$ $CD28^-$ T-cells has also been documented in other inflammatory states, such as HIV infection, systemic lupus erythematosus, rheumatoid arthritis, and Wegener granulomatosis[32].

Senescence decreases the production rate of immature bone marrow B-cells; however, peripheral B-cell numbers seem to be maintained due to a reduced turnover of mature B-cells[3]. Besides, quantitative and qualitative antibody response is reduced in the elderly[31].

Uremic toxins induce oxidative stress and inflammation, which alters innate and adaptive immune systems, changes that weaken immunity in chronic kidney disease (CKD) patients[33]. Studies on T-cells in end-stage chronic renal disease patients documented that their telomere shortening showed an immunological age that was advanced by 20 years compared to their chronological age[33]. Neither hemodialysis nor peritoneal dialysis has shown to reverse telomere shortening in CKD patients[33]. A uremic environment also causes epigenetic changes that may contribute to aging; for instance, methylation of the *Klotho* gene is initiated by oxidative stress in CKD patients, and leads to a syndrome that resembles human aging. Even so, despite that kidney transplantation solves a uremic proinflammatory environment, it is not able to reverse epigenetic changes[33].

This senescence process could be pharmacologically modified since it has been documented that bardoxolone can attenuate T-cell aging in advanced CKD patients, but it has the inconvenience of increasing cardiovascular diseases. Another alternative that has been reported is to stimulate T-cell function using interleukin 7 in this group[33].

Immunosenescence in allograft recipients seems to be useful since it can downturn immune reactivity against the allograft or even induce tolerance to the donor antigens. On the other hand, it promotes a particular phenomenon in grafts known as inflammaging, which is the term coined for explaining the impact of donor advanced age on graft immunogenicity[4]. In this sense, chronic subclinical cytomegalovirus infection could be the main accelerator of senescence, particularly in transplant patients on immunosuppressant drugs, since it represent a persisting challenge to the immune system[4,30].

IMMUNOSUPPRESSANT DRUGS

It is important to note that a kidney graft always suffers a fast senescence rate compared to a native kidney, since the development of severe functional reduction

(glomerular filtration rate around 10 ml/min/1.73 m²) would take a longer time in the native organ: about 120 years. Thus, the aging process suffered by a kidney graft seems to be a sort of progeria or premature senescence[34,35].

Even though immunosuppressant drugs play a central role in organ transplantation, their role in graft senescence is also known. The described aging mechanisms induced by immunosuppressant are the following :

– Cyclosporin A (calcineurin inhibitor) nephropathy and renal aging share some histopathological findings such as renal fibrosis and tubular atrophy[36]. This drug significantly increases the rate of cell apoptosis, p16^{INK4a} and p21 expression, telomere shortening, decreased *Klotho* expression, and intra-renal renin-angiotensin system (RAS) activation, all changes related with senescence[11,29]. Moreover, it has been proposed that cyclosporine downregulates *Klotho* via direct toxicity or via RAS activation, and that cyclosporine-induced graft aging is induced by increasing oxidative stress[13]. Besides, losartan treatment restores *Klotho* expression in cyclosporine-induced renal injury[11].

– The BENEFIT study has shown that senescent, CD4$^+$/IL-17A$^+$, p16 positive cells, and interstitial fibrosis were significantly increased in graft biopsies among patients on cyclosporin A compared to those on belatacept[29].

– Telomerase, the enzyme which repairs telomere shortening, is inhibited in most human differentiated cells because of the repression of the *hTERT* gene, and consequently these cells present telomere erosion, senescence, and finally apoptosis. It has been documented that cyclosporine and FK-506 dose-dependently block hTERT and promote telomerase inhibition, and consequently premature aging of T-cells[1,17,18].

– Prednisone and mycophenolate mofetil can also induce T-cell senescence[1].

– Food restriction without malnutrition prolongs the lifespan of animal species. Since the mammalian target of rapamycin (mTOR) enzyme acts as a sensor of energy supply, it could have a role in the life-prolonging effect of caloric restriction. This could also explain why rapamycin (mTOR) inhibitor delays aging and prolongs lifespan in experimental models[14].

– Some evidence suggests that rapamycin (sirolimus) could cause an increase of *Klotho* gene expression, inhibiting FGF23 coreceptor by tubular cells[14]. However, in the presence of cyclosporine-induced renal damage, rapamycin can accelerate it by enhancing oxidative stress[13].

– Immunosuppressive treatment predisposes to viral infection, which can induce aging[1,4,30].

KIDNEY FROM OLDER DONORS

Kidneys from older donors usually show worse graft survival: transplanted kidneys from elderly donors generally have a projected half-life significantly lower (5 years) compared to kidneys from young donors (10 years), and this phenomenon has been attributed to the presence of a reduced number of glomeruli in the aged kidneys[3,4]. However, there are studies that found no significant difference between older and younger donors in allograft survival[8].

Since the transplant procedure can induce telomere shortening, and telomeres are already shortened in aged grafts, it is conceivable that older kidney transplantation usually has a worse course compared to younger kidney transplantation[3].

Besides, independent of telomere shortening, older grafts have an impaired capacity to handle stress, control inflammation, and repair structural damage[37].

Additionally, older donors are more likely to have hypertension, microvascular renal damage, and glomerulomegaly with associated hyperfiltration, and these preexisting structural abnormalities could amplify external insults such as glomerular ischemia from superimposed arteriolar hyalinosis from calcineurin inhibitors, hypertension, or dyslipidemia[37]. Moreover, senile tissue injury facilitates immune recognition and a subsequent increased immunogenicity of the old donor kidney[4,9,37]. This is one of the main reasons for proposing to transplant an older kidney into an older recipients since it may optimize the outcome, since the less vigorous alloresponses of old recipients may counterbalance the increased immunogenicity of old

grafts. Conversely, it has been documented that old kidneys that are transplanted into young recipients show the highest rejection rates, while this phenomenon is blunted when aged organs are transplanted into old recipients[9,38]. Another reason for an "old-for-old program" strategy is that older grafts may be sufficient for handling metabolic demands of older recipients[4].

It is worth taking into account that defining as elderly an individual older than 64 years of age is not a biological concept but a social one. It is known that the aging process in the native kidney starts around 35 years of age, so it should be realized that a young adult donor (55 years old) may in fact be providing an old organ since it has already started its aging process 20 years ago, with the clinical consequences that this will have on graft evolution when other variables start playing a role, such as a CKD setting and the use of immunosuppressant drugs.

The following are strategies described as potentially useful for ameliorating the senescence process in kidney transplantation:

– Belatacept, an indolamine 2,3-dioxygenase immune modulator, induces tryptophan deficiency, and since tryptophan deficit contributes to suppress lymphocyte apoptosis, this drug leads to a less deleterious effect on senescent inflammatory cells[29].

– Kidney transplant patients usually show increased oxidative stress and reduced anti-oxidative markers, suggesting that oxidative stress plays a crucial role in the progression of graft damage. This oxidative stress generates free radicals, which induce DNA breaks and telomere erosion. Thus, the use of antioxidants in kidney preservation solutions could be helpful in preventing this sort of graft damage and influence long-term function[3].

CONCLUSIONS

Renal graft senescence is a complex process, and its better understanding will help nephrologists to improve its management in order to achieve a longer graft survival. This therapeutic approach would be very useful particularly in grafts obtained from older donors whose functional durability would be significantly increased.

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Aging and Pulmonary Fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis is a chronic, progressive, and usually fatal lung disorder of unknown etiology. The disease likely results from the interaction of genetic susceptibility architecture, environmental factors such as smoking, and an abnormal epigenetic reprogramming that leads to a complex pathogenesis. Idiopathic pulmonary fibrosis occurs in middle-aged and mainly elderly adults, and in this context age has emerged as its strongest risk factor. However, the mechanisms linking it to aging are uncertain. Recently, nine molecular and cellular hallmarks of aging have been proposed: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. In this review, we provide an overview of these molecular mechanisms and their involvement in the pathogenesis of idiopathic pulmonary fibrosis, while emphasizing that the studies on this disease are few and the findings are not definitive. (REV INVES CLIN. 2016;68:75-83)

Key words: Aging. Lung fibrosis. Senescence. Telomeres.

INTRODUCTION

Biological lung aging is characterized by structural changes and progressive loss of physiological integrity, leading to impaired function[1]. Although the mechanisms that contribute to the aging process are uncertain, nine putative hallmarks associated with the aging phenotype have recently been proposed[2]. However, in what way and magnitude they participate in the aging lung is unknown.

FUNCTIONAL AND STRUCTURAL MODIFICATIONS OF THE LUNGS AND THORAX DURING AGING

In general, "normal aging" is characterized by narrowing of the intervertebral disk spaces and increased prevalence of hyperkyphosis. In fact, 20-40% of older adults present an excessive curvature of the thoracic spine[3]. In addition, there are changes in the intrinsic function of the muscles, which are associated with reduced inspiratory

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Figure 1. Expiratory high-resolution computed tomography scan revealing inhomogeneous lung attenuation due to air trapping identified by the presence of areas of low attenuation next to regions with normal attenuation (arrows).

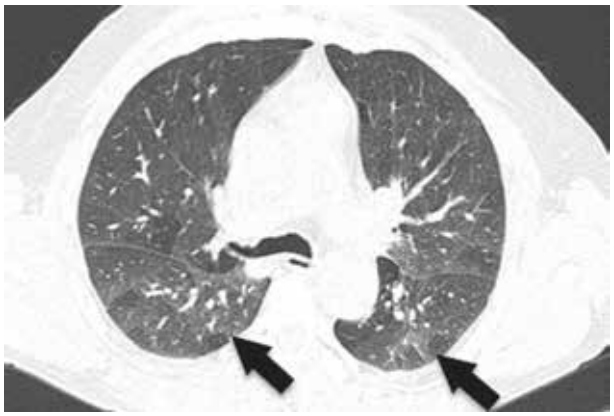
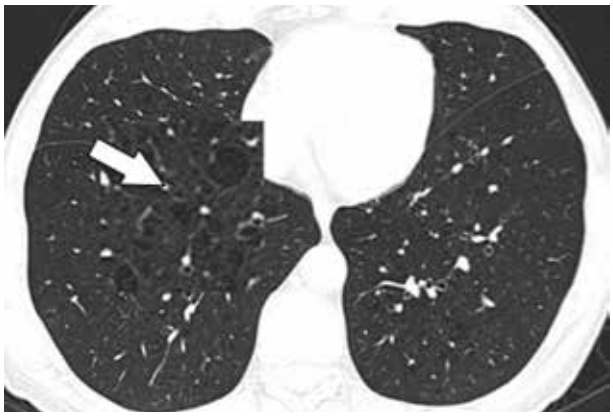


Figure 2. Early stage of centriacinar emphysema in a 73-year-old asymptomatic individual. High-resolution computed tomography demonstrates numerous tiny low attenuation areas throughout the lung field (arrow).



and expiratory respiratory muscle strength. This process, together with a decrease in the mitochondrial adenosine triphosphate (ATP) reserves, contribute in older individuals to having difficulties in sustaining a sudden rise in metabolic demand, increasing the risk of respiratory failure in acute lung diseases.

A common finding with aging is a decrease of lung elasticity[4]. This affects the small airways and alveolar septa, and may explain at least two frequent observations in the elderly. The first is a premature collapse of the peripheral airways, which provokes the so-called "air-trapping" that is more evident during expiration (Fig. 1). The other is the increase in size of the alveolar ducts and alveoli, which was previously called "senile emphysema", although this is not an appropriate term since it lacks the characteristic destruction of the alveolar walls seen in emphysema (Fig. 2). Nevertheless, the alveolar over-distention results in an increase of the residual volume of about 5-10% per decade[5].

Other studies performed in older individuals (> 75 years old) without known respiratory disease have also reported the presence of reticular opacities (suggestive of fibrosis), as well as airway dilation, bronchial thickening, and bronchiectasis when compared with younger (< 55 years old) subjects[6]. Changes in the airways are associated with dysfunction of the mucociliary escalator, decreased capacity to clear mucus and particles from the lungs, and a reduction in cough strength[7]. We have found similar alterations in an ongoing study on aging lung in asymptomatic individuals (Selman, et al., unpublished results) (Fig. 3).

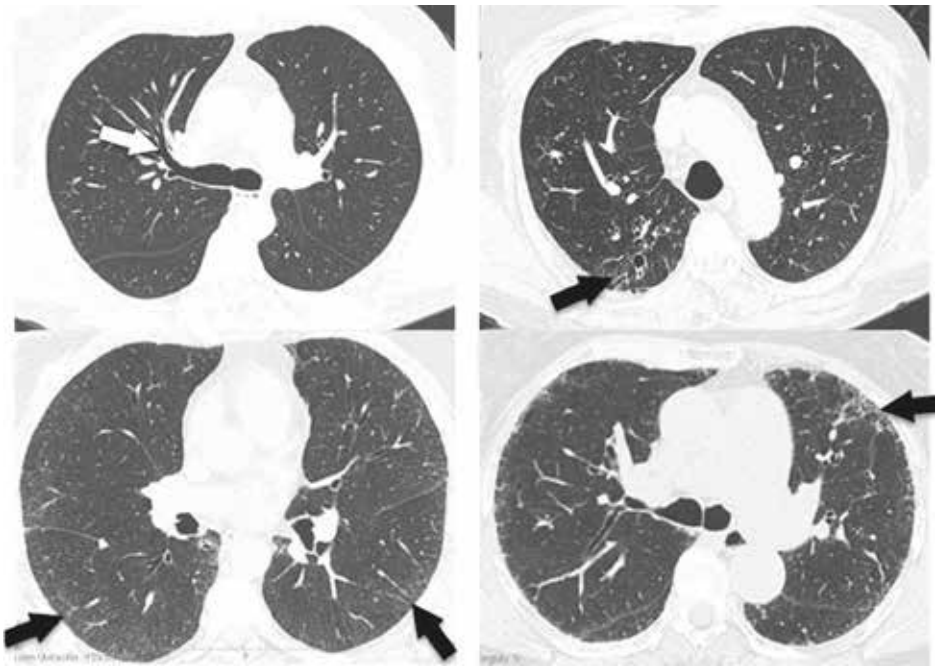
Physiological age-related pulmonary changes are characterized by a decrease of approximately 30 ml each year in forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC)[8,9]. Likewise, the mentioned increase in the closing volume by the premature collapse of the small airways, combined with diverse age-related changes in the pulmonary circulation, result in a heterogeneous distribution of the ventilation/perfusion ratio. This, together with a decrease in the diffusing lung capacity for carbon monoxide (DLCO), causes an age-related decline in the arterial tension for oxygen (PaO_2)[10-12].

LUNG DISEASES ASSOCIATED WITH AGING

There are two types of lung disorders associated with aging: those that may occur at any period of life but whose severity is affected by aging, and those that occur virtually only in old people. Among the first type, asthma, obstructive sleep apnea, and pulmonary edema in the setting of congestive heart failure are some of the most common[13,14]. Likewise, decreased respiratory muscle strength, attenuated cough, dysfunction of mucociliary clearance, and altered immune response increase the risk for lung infections in elderly patients[13].

By contrast, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) are two diseases usually diagnosed in individuals over 50 years old and whose incidence and prevalence increase remarkably with age. Thus, the prevalence of COPD in persons aged 65 years and older in the

Figure 3. High-resolution computed tomography showing several abnormalities detected in elderly asymptomatic subjects. **A:** Central airway dilation. **B:** Bronchiectasis. **C:** and **D:** Peripheral, subpleural septal thickening (arrows).



general population is at least 10-15%[15]. The real incidence and prevalence of IPF are uncertain, but in the USA it has been reported that the incidence is about 10 per 100,000 persons per year, which increases to approximately 90 per 100,000 per year in people aged 65 years and older[16,17].

IDIOPATHIC PULMONARY FIBROSIS: THE INFLUENCE OF AGING

Idiopathic pulmonary fibrosis is a progressive, irreversible, and usual fatal lung disorder of unknown etiology[18]. It has been proposed that the disease is triggered by an aberrant activation of alveolar epithelial cells (AEC), which in turn induces the migration, proliferation, and activation of fibroblasts/myofibroblasts, leading to the exaggerated accumulation of extracellular matrix and the subsequent destruction of the lung architecture[19]. As mentioned before, IPF occurs in middle-aged and mainly elderly adults, suggesting a mechanistic link between chronological age and this disease. However, the biopathological mechanisms that link aging with the pathogenesis of IPF have not been elucidated.

Recently, nine putative cellular and molecular hallmarks were proposed to contribute to the aging processes and aging phenotype[2]. Although studies in IPF are few, almost all of these hallmarks have been examined and results suggest that an accelerated aging process occurs in this disease.

GENOMIC INSTABILITY

Age-dependent accumulation of DNA damage is a well-recognized component of the aging phenotype[2]. Several studies have reported the presence of genomic instability in IPF patients[20-22]. The incidence of microsatellite instability (MSI) and loss of heterozygocity (LOH) were determined in cytological sputum specimens from 26 IPF patients and 26 matched controls using 10 highly polymorphic microsatellite markers[20]. Fifty percent of the patients displayed genetic alterations, either MSI or LOH. The most commonly affected microsatellite markers were THRA1 and D8S133. Subsequently, a one-base-pair deletion was detected in the polyadenine tract in exon 3 of the transforming growth factor (TGF)-beta RII receptor gene in AECs isolated by microdissection from IPF lungs. Furthermore, in these areas, low expression of the receptor was confirmed[21]. Finally, 40 microsatellite markers were evaluated in 52 sputum/venous blood DNA pairs from IPF patients[22]. Twenty specimens (38.5%) exhibited LOH in at least one of the examined loci; LOH

was observed in microsatellite DNA markers located in MYCL1, FHIT, SPARC, p16Ink4, and TP53 genes. Taken together, these findings indicate that genetic instability likely affecting genes involved in critical cellular pathways is a relatively frequent phenomenon that could account for the pathogenesis of IPF.

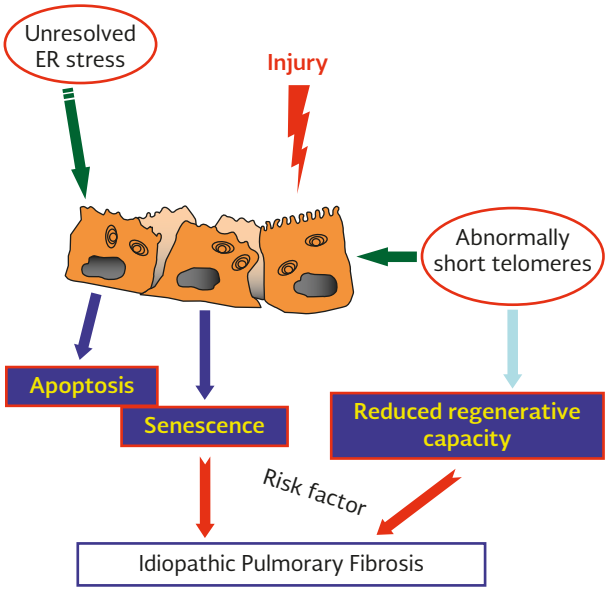
TELOMERE ATTRITION

Telomere shortening is considered one of the most influential mechanisms of cellular aging. When telomeres become critically short, they activate a DNA damage response that provokes cellular senescence or apoptosis[23]. Abnormal telomere shortening has been associated with several progressive disease phenotypes that share the short telomere defect as a driving mechanism[23]. Telomerase mutations cause approximately 20% of the cases of familial IPF (identified by the presence of two or more individuals in a family having pulmonary fibrosis), and all of these patients characteristically have very short telomeres[24-26]. Furthermore, 20-30% of patients with sporadic IPF that do not have mutations in telomerase components displayed telomere lengths less than the 10th percentile when compared with control subjects[27]. The mechanisms by which telomere defects contribute to IPF are uncertain. It has been proposed that telomerase mutations (familial IPF) or exaggerated proliferative response (sporadic IPF) lead to telomere shortening in the alveolar epithelium and that this is critical for the development of the disease. A recent study supports this notion[28]. In this work, late-generation telomerase-null mice induced by deleting telomeric repeat-binding factor 2 (Trf2) was generated, and in this conditional mutant model, where telomere dysfunction was restricted to type 2 AECs (AEC2), the stem cell function of this subpopulation was impaired, leading to senescence. Moreover, when telomere dysfunction was induced in purified adult AEC2s, *ex vivo* cells survived but remained senescent[28]. These results indicate that AEC2-dependent telomere dysfunction and senescence limit alveolar repair and can signal mesenchymal abnormalities (Fig. 4).

CELLULAR SENESCENCE

Cellular senescence has been considered a critical event in biological aging. It refers to a permanently arrested state of cell growth together with the achievement of

Figure 4. Alveolar epithelial cells play a critical role in the pathogenesis of idiopathic pulmonary fibrosis. Unsolved endoplasmic reticulum stress and extreme shortening of telomeres may lead to epithelial cell death or senescence, and the senescence-associated secretory phenotype characterized by the upregulation of genes encoding a complex pro-inflammatory and profibrotic transcriptional response. ER: endoplasmic reticulum.



the senescence-associated secretory phenotype, characterized by the release of a variety of inflammatory, growth-regulating, and tissue-remodeling factors[2,29].

Recently, AEC senescence was revealed in IPF lungs[30]. In this study, strong staining of β-galactosidase, a marker of senescence, and p21/waf-1, a senescence-associated cyclin-dependent kinase inhibitor, was observed in the lung epithelium. These results were confirmed in a second study where nuclear staining of p21 was clearly demonstrated only in epithelial cells covering actively fibrosing lesions, while β-Gal-positive staining was observed in epithelial cells covering fibroblastic foci[31]. Alveolar epithelial senescence, likely related to shortening of telomeres, may contribute to the high secretory profile exhibited by these cells in IPF.

On the other hand, studies on fibroblasts have given elusive results. Recently, a study demonstrated that fibroblasts within fibroblastic foci of IPF lungs show features of senescence. Expression of p16 and p21 was seen in fibroblasts within the foci and in the overlying epithelial cells[32]. Moreover, fibroblast expression of NADPH oxidase-4 (Nox4) was increased in IPF lung

fibroblasts, and the use of a specific inhibitor attenuated βgal activity, suggesting that Nox4 contributes to cellular senescence of IPF fibroblasts. More recently, a study showed that IPF fibroblasts displayed an accelerated entry to replicative senescence, accompanied by an accumulation of senescent cells with features of myofibroblasts characterized by high expression of alpha smooth muscle actin (α-SMA)[33].

There is also some "systems senescence" in IPF patients, e.g., immune senescence or endocrine senescence, which may contribute to the development or progression of IPF. For example, a marked downregulation of CD28 on circulating CD4 T-cells has been found in IPF patients compared with age-matched controls[34]. CD28 is a major co-stimulatory molecule responsible for the optimal activation of naive T-cells. It is also involved in proliferation, survival, and glucose metabolism. The T-cells lose CD28 expression with age, often taken as a hallmark of aging human T-cells[35].

Deterioration of the endocrine system also occurs during aging and is thought to contribute to increased susceptibility to aging-associated diseases. In this context, we evaluated the blood levels of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S), the most abundant adrenal steroids in humans. Under physiological conditions, DHEA/DHEA-S reach a peak between the ages of 25 and 30 years and thereafter gradually decline so that, by the age of 60, the concentrations are only 10-20% of corresponding values in young adults[36]. We found that IPF patients had a disproportionate decrease in the circulating levels of DHEA-S compared with age-matched controls. Moreover, DHEA displayed a strong antifibrotic effect on fibroblasts, affecting migration, proliferation, differentiation to myofibroblasts, collagen synthesis, and survival, indicating that its exaggerated decline may participate in the pathogenesis of the disease[36].

MITOCHONDRIAL DYSFUNCTION

Mitochondria play a key role in cellular homeostasis, bioenergetic capacity, and longevity since they are the highest producers of ATP and regulate programmed cell death. Aging is associated with the expansion of dysfunctional mitochondria, with alterations in mitochondrial dynamics and quality control processes resulting from an imbalance of fission and fusion events and increased production of reactive oxygen species (ROS)[37]. Mitochondrial DNA (mtDNA) is damaged by ROS generated during oxidative metabolism, and the accumulation of damaged mtDNA and decreased mitophagy result in loss of fidelity in the synthesis of new mitochondria proteins, leading to senescence and aging[2].

Excessive production of ROS and disruption of the oxidant/antioxidant balance in the lung have been found in IPF[38]. In the expired breath condensate, the concentrations of H_2O_2 and 8-isoprostane, which are markers of oxidative stress, are usually increased in IPF patients compared with normal controls, indicating high levels of oxidative stress[39]. Likewise, a marked reduction of levels of glutathione, a major antioxidant molecule, has been observed in bronchoalveolar lavage, sputum, and plasma of patients with IPF[40,41].

Recently, a study demonstrated that AEC2 of IPF lungs exhibit an age-related mitochondrial dysfunction with altered structure and impaired mitophagy[42]. Deficiency of PTEN-induced putative kinase 1 (PINK1) was identified as a fundamental mechanism leading to accumulation of dysfunctional mitochondria and, moreover, the mitochondrial phenotype observed in IPF lungs and susceptibility to lung fibrosis was recapitulated in an animal model of aging and PINK1 deficiency. Importantly, several chronic degenerative diseases associated with aging, such as Parkinson's disease and neuropsychiatric disorders, present mutations or deficit of PINK1 and show swollen and dysfunctional mitochondria and poor mitophagy, indicating that this may be a common phenomenon in aging-associated diseases.

LOSS OF PROTEOSTASIS

Aging and some aging-related diseases are associated with impaired proteostasis. Protein homeostasis involves mechanisms for the stabilization of correctly folded proteins and mechanisms for the degradation of proteins by two principal proteolytic systems implicated in protein quality control: the autophagy-lysosomal system and the ubiquitin-proteasome system[2]. There is a strong body of evidence indicating that aging is associated with disturbed proteostasis, which may contribute to age-associated disorders. Furthermore, maintenance of appropriate autophagic activity prevents or slows down the functional failure

associated with cellular proteotoxicity and accumulation of intracellular damage in aging[43].

Recent work has approached the putative role of autophagy in IPF, while studies on the ubiquitin system are scant.

Autophagy is a complex process involving multiple proteins and steps, including the formation of an initiation complex and development of a double-membrane phagophore; elongation of the membrane and completion of an autophagosome vesicle around cargo; lysosomal fusion; dissolution of the inner membrane allowing hydrolases to degrade the cargo; and recycling of the components[44].

In the first approach in IPF, it was reported that LC3-II levels (commonly used as a marker of autophagy) were significantly lower in whole tissue homogenate of lungs from patients with IPF compared with control lungs. In experimentally induced lung fibrosis, it was shown that the inhibition of mTORC1, a primary modulator of autophagy, with rapamycin attenuated the fibrotic response[45]. In this study, they also found that inhibition of autophagy potentiated fibroblast to myofibroblast differentiation and activation. A subsequent study, using biochemical evaluation of *in vitro* models, demonstrated that autophagy inhibition is sufficient to induce acceleration of epithelial cell senescence and myofibroblast differentiation in lung fibroblasts[31]. More recently it was shown that an aberrant PTEN/Akt/mTOR axis desensitizes IPF fibroblasts from polymerized collagen-driven stress by suppressing autophagic activity, which produces an IPF fibroblast phenotype resistant to apoptosis in collagen[46].

Most studies regarding the role of autophagy in lung fibrosis have focused on fibroblasts. A more recent work suggests that epithelial cells may also be affected. In an experimental model induced by bleomycin, it was shown that Atg4b-deficient mice exhibited reduced autophagy and a significantly higher inflammatory and fibrotic response compared with the wild-type littermate. Importantly, the study found that Atg4b disruption resulted in increased apoptosis, affecting predominantly alveolar and bronchiolar epithelial cells[47]. These findings indicate that autophagy protects epithelial cells against bleomycin-induced stress and apoptosis, and participates in the attenuation of the inflammatory and fibrotic responses.

Importantly, evidence suggests that there is an age-related decline in autophagy and selective targeting of mitochondria for autophagic degradation that enhances the lung fibrotic response in experimental models[48]. This reduction seems to be exaggerated or accelerated in IPF, a natural aging-associated human fibrosis.

Oxidative stress, endoplasmic reticulum (ER) stress, and hypoxia, all mechanisms that participate in the pathogenesis of IPF, are well-known inducers of autophagy. However, this protective mechanism is dysfunctional, likely contributing to the pathobiology of the disease.

The ubiquitin-proteasome system is the major degradation pathway for short-lived proteins in eukaryotic cells. Its relevance for preservation of protein homeostasis in the lung is emerging for chronic lung diseases[49]. In this context, inhibition of this system by specific proteasome inhibitors has been shown to provide antifibrotic effects in the mouse model of bleomycin-induced lung damage[50].

However, the regulation of proteasome function in IPF has not been explored in detail. Recently, a study showed that the proteasome is activated in the process of TGF-β-induced human myofibroblast differentiation[51]. The activation resulted from increased formation of 26S proteasomes. In IPF lungs, the expression of the subunit Rpn6 was upregulated specifically in myofibroblasts and hyperplastic AECs overlying fibroblast foci. Elevated levels of K48-polyubiquitin protein conjugates in these cells and the positive correlation of whole lung Rpn6 protein levels with K48-polyubiquitinated proteins suggest that activation of ubiquitin-dependent protein degradation by the 26S proteasome may be a pathologic feature of fibrotic remodeling occurring specifically in IPF[51].

STEM CELL EXHAUSTION

The balance between stem cell self-renewal and differentiation is critical to orchestrate tissue homeostasis and the response for repair/replacement of damaged tissues. In this context, a major hallmark of aging is a reduced ability to regenerate, which has been associated with a decline in proliferative activity, impaired function, and exhaustion of tissue-specific stem and progenitor cells[2]. There is an emerging

body of evidence indicating that reduced function of adult stem cells plays an important role in the development of age-related diseases[52]. So far, no studies in IPF have been published. In a recent report, bone marrow-derived mesenchymal stem cells (B-MSC) derived from old animals were found to display a remarkable downregulation of multiple chemokine receptors such as CCR7, CX3CR1, and CXCR5 as well as other genes involved in migration[53]. When lungs were injured with *Escherichia coli* lipopolysaccharide, aged endogenous B-MSCs not only failed to migrate appropriately to the injury site, but once there they also failed to produce enough of the anti-inflammatory agents that characterize their younger forms. Interestingly, there were similar differences between B-MSCs obtained from young and aged human individuals; that is, old cells showed a downregulation of cytokine receptors, decrease in activation, and migration.

DEREGULATED NUTRIENT-SENSING

Insulin-like growth factor (IGF-1) and insulin signaling are known as the "insulin and IGF-1 signaling" and represent the most conserved aging-controlling pathway in evolution[2]. Among its multiple targets are the mammalian target of rapamycin (mTOR) complexes, which are also involved in aging and recently have been implicated in lung fibrosis. For example, in a recent work, aberrant mTOR signaling activation was provoked in AECs using conditional *Tsc1* knock-down mice that were then injured with bleomycin[54]. Mice with increased mTOR activation exhibited high mortality and exaggerated lung fibrosis compared with control mice. Moreover, mTOR inhibition with rapamycin rescued bleomycin-mediated lung injury and fibrosis. These findings were associated to decreased autophagy that, as mentioned, seems to contribute to abnormal repair and fibrosis.

Supporting the role of mTOR complexes in the fibrotic response, a recent study in IPF lung fibroblasts demonstrated that TGF-β, a major profibrotic mediator, induced the Rictor component of mTORC2, which led to Akt activation[55]. Moreover, the use of a specific inhibitor of the active site mTOR attenuated the expression of profibrotic matrix-regulatory proteins in TGF-β-stimulated IPF fibroblasts and inhibited the fibrotic response in a murine bleomycin lung model[55]. Overactivation of mTOR has been found in fibroblast foci and alveolar epithelial cells of IPF lungs[54,56].

EPIGENETIC ALTERATIONS

Epigenetic mechanisms are heritable changes in gene activity that are independent of alterations in the underlying DNA sequence. In a more extensive definition, epigenetic includes the set of covalent modifications to DNA, posttranslational modifications to histones, and the regulatory effect of non-coding RNAs that influence the expression of genes and the structure of chromatin. All these epigenetic processes do not act independently, but strongly interact to form a complex regulatory system that can dynamically adjust the gene expression. Epigenetic marks are remodeled and may actively modulate the processes of aging.

DNA METHYLATION AND IDIOPATHIC PULMONARY FIBROSIS

DNA methylation is a covalent modification that occurs on cytosine, mostly located in CG dinucleotides (CpG). Cytosine methylation primarily happens in CpG-rich sequences, dubbed as CpG islands, resulting in the constitutive silencing of chromatin regions.

Aging is characterized by hypomethylation of sites outside promoter CpG islands, while CpG islands near promoters are typically hypermethylated, and there is some evidence indicating that these modifications in DNA methylation may be a sensor for both chronological and biological age[57].

Studies in IPF are scant and initially focused on putative meaningful candidate genes. Thus for example, Thy-1 (CD90), an important regulator of fibroblast behavior, is absent in myofibroblasts within fibroblastic foci in IPF, and its downregulation is mediated at least partially by the hypermethylation of the promoter[58]. Likewise, different levels of methylation of three CpG islands in the promoter of α-SMA in fibroblasts and myofibroblasts correlate with the levels of expression of this gene[59]. On the other hand, we have demonstrated that IPF fibroblasts have reduced expression of the proapoptotic p14ARF attributable to promoter hypermethylation, suggesting that epigenetic mechanisms may underlie their resistance to apoptosis[60].

Global methylation and gene expression patterns have been recently examined in IPF lungs[61]. By comprehensive high-throughput arrays, 4.6 million CpG sites

distributed across the human genome as well as the gene expression changes were examined in 94 IPF lungs and 67 controls. Over 2,000 differentially methylated regions associated with 1,514 unique genes were identified, with the majority of the methylation changes located outside of promoter CpG islands. Functional analyses identified several enriched canonical pathways that have been implicated in the pathogenesis of IPF, including CXCR4 signaling, thrombin signaling, Wnt/β-catenin signaling, and epithelial adherens junction signaling. Analysis of binding motifs in promoters revealed overrepresentation of regulators of lung development, specifically, β-catenin, GLI1, and FOXC2; this is important since the upregulation of developmental pathways is involved in the aberrant activation of epithelial cells[62]. These findings support the notion that several biologically relevant methylation-expression changes may contribute to the development of IPF.

NON-CODING RNA AND IDIOPATHIC PULMONARY FIBROSIS

Two main sub-groups of regulatory-type non-coding RNA (ncRNA) have been described: the short ncRNAs (< 30 nucleotides long), that include microRNAs (miRNA), short interfering RNAs (siRNA), and piwi-interacting RNAs (piRNA); and the long ncRNAs that contain over 200 nucleotides and seem to control genome activity at the chromatin level.

Epigenetic deregulation of ncRNAs, primarily miRNAs, has been observed in IPF. In fact, different studies have shown that approximately 10% of miRNAs are deregulated and an imbalance between profibrotic and antifibrotic miRNAs are thought to be linked to the development or progression of IPF[63,64]. The downregulated miRNAs include miR-326, let 7d, miR-26a, miR-29, miR-200, and miR-17~92, while miR-21, miR-154, 199a-5p, and miR-145 are upregulated. In general, all these miRNAs play roles in the TGF-β1 signaling pathway, fibroproliferation, lung epithelial cell development, and epithelial to mesenchymal transition, and their deregulation results in the facilitation of many profibrotic processes.

It is important to emphasize that all these epigenetic mechanisms are integrated through complex crosstalk pathways and feedback loops. For example, an association between aberrant DNA methylation and miRNA expression has been recently identified in IPF[65]. Thus, increased DNA methylation in the promoter of the miR-17~92 clusters silence its expression, which in turn results in the upregulation of genes strongly related to the fibroproliferative response and the fibroblast phenotype in IPF.

Finally, whether some of the mentioned epigenetic changes observed in IPF are related to aging is uncertain. It has been proposed that there is a stochastic age-related DNA methylation drift, which is bidirectional (both hyper- and hypomethylation), is not uniform across the genome, and is quite variable between individuals of the same age[66]. It is tempting to think that in few of them, the drift particularly affects genes whose up- or downregulation results in a profibrotic reprogramming.

CONCLUSIONS

Aging is a multifaceted process that results in progressive decline in homeostasis and increased risk of disease or death. Incidence and prevalence of IPF increase remarkably with aging. Before 50 years of age, IPF is rare, but over 60 years old, the prevalence may be as high as 300/100,000, indicating a strong link between aging and IPF. Most of the hallmarks of aging seem to be involved in the development or progression of IPF. However, studies to date were performed in small cohorts and have produced heterogeneous results. In the future it will be necessary to integrate the genetic and epigenetic data to identify regulatory pathways associated with aging and identify which of them may be implicated in the pathogenesis of IPF.

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The Role of Immunosenescence in the Development of Age-Related Diseases

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ABSTRACT

Aging is a complex phenomenon leading to numerous changes in the physiological systems of the body. One of the most important changes, called immunosenescence, occurs in the immune system. Immunosenescence covers changes in the innate and the adaptive immune systems and is associated with a low-grade inflammation called inflammaging. Aging, likely via inflammaging, is also associated with the emergence of chronic diseases including cardiovascular and neurodegenerative diseases, cancer, and diabetes mellitus type 2. The origin of this inflammaging is not known with certainty, but several concurrent contributing factors have been suggested, such as aging-associated changes in the innate and adaptive immune response, chronic antigenic stimulation, the appearance of endogenous macromolecular changes, and the presence of senescent cells exhibiting a senescence-associated secretory phenotype. A better understanding of the multiple biological phenomena leading to these diseases via the immunosenescence associated with inflammaging provides a powerful target for interventions to increase the healthspan of elderly subjects. (REV INVES CLIN. 2016;68:84-91)

Key words: Inflammaging. Immunosenescence. Chronic diseases.

INTRODUCTION

Aging is a complex biological and physiological process[1,2]. The exact cause of aging is not known. However, we know that with aging, the incidence and prevalence of chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative diseases are increasing[3,4]. Indeed, the most important risk factor for the occurrence of these diseases is age. The relationship between aging and these age-related diseases is still actively being searched for. Recently, a new concept called "geroscience" was proposed to understand the putative role of aging in the appearance of age-related diseases and seeks to develop novel multi-disease preventative and therapeutic approaches[5-7]. The corollary of this concept is "healthspan" or a substantial extension in healthy life expectancy. This states that by decreasing/decelerating the rate

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of aging we can increase the time spent in health before the appearance of these age-associated diseases[6]. In this review we will describe the age-related changes, especially in the immune system, which can lead to the development of these age-related chronic diseases.

AGE-RELATED CHANGES IN THE IMMUNE SYSTEM

Aging is associated with several changes in the physiology of many organs and systems underlined by molecular, cellular, tissue, and organismal changes[1]. In a recent work, nine hallmarks of aging were described[8]. Among these hallmarks, what emerged very recently as one of the most determinant is changes in the immune system[9-12]. These changes in the immune response are presently called "immunosenescence". This concept is not clearly defined and recent research questioned many paradigms linked to this concept[10-13]. In spite of this debate, numerous experimental data support the changes in the immune system with aging, even in humans.

Immunosenescence

This concept suggests that the immune system as a whole is aging, not in one block, but with certain parts aging more than others. The immune response is composed by two distinct, but closely interrelated parts: the innate and the adaptive parts[9-12]. It is overwhelmingly recognized that the immune changes occurring with aging affect the adaptive part. However, recently it was also recognized that there are substantial changes in the innate immune system[9,10].

The innate immune system is composed of several cells, including neutrophils, monocytes/macrophages, and dendritic cells. In spite of the fact that natural killer (NK) cells are part of the innate-like lymphocyte group, we will discuss their changes in this paragraph.

Neutrophils are the first to arrive at the site of aggression. Their lifespan is quite short as, if unstimulated, they die by apoptosis, but their lifespan can be increased by pro-inflammatory stimuli such as lipopolysaccharide (LPS)[14,15]. Indeed, the number of neutrophils was reported to be relatively high in the elderly, even if within the normal range. The effector functions of neutrophils were altered after several receptor stimulations by their specific ligands, such as LPS, formyl-methionyl-leucyl-phenylalanine (FMLP), or granulocyte-macrophage colony stimulating factor (GM-CSF) including chemotaxis, intracellular killing, and respiratory burst resulting in free radical production[16-21]. Interestingly, in most studies the phagocytosis and adherence did not show age-related alterations. It is of note that the changes in these effector functions can be, on the one hand, explained by the activation of these neutrophils already in the quiescent state manifested by the increased free-radical production, cytokine production, and metalloproteinase production, concomitantly to the sustained nuclear factor kappa B (NF-kB) activation[9,22-24], and on the other hand by the alteration of the signaling pathways[25]. The most important signaling pathway is PI3K, which has been found to be altered in neutrophils with aging[26].

The monocyte/macrophage lineage also shows age-related changes, although interestingly this is much less studied in humans. However, the results indicate that most of the effector functions of these cells are decreased, including cytotoxicity, intracellular killing, and antigen presentation[24,27,28]. The recently discovered toll-like receptor (TLR) functions are also altered in these cells. Some monocyte subpopulations, mainly with an inflammatory phenotype such as $CD14^+CD16^+$, are increased[29]. This is manifested by the increase of pro-inflammatory cytokine production at the quiescent state, while they are decreased during stimulation. Similar changes were observed in plasmacytoid and myeloid dendritic cells, resulting in impaired antigen presentation and $CD4^+$ T-cell activation[30].

The NK cells have important killing functions toward virus-infected and cancerous cells[9,31]. Two distinct populations exist considering the CD56 cell surface markers. With aging, the CD56 bright subpopulation decreases while the CD56 dim subpopulation increases. On a single cell basis, the cytotoxic activity of NK cells decreases, but their greater number compensates for this decrease. Together these changes observed in the innate system indicate that with aging there is a basal activation state, which manifests itself by increased pro-inflammatory mediators concomitantly with decreased receptor signaling and effector function decrease[9,24,25,32-34]. The question arises, why is that so? The origin of these changes is not well understood; however, a chronic low-level stimulation

by infectious agents, some related to gut microbiota, can be postulated. The barriers (especially in the gastrointestinal system) are more permeable, especially in the presence of low-grade inflammation, and many substances can be found in the circulation or in tissues even if at the origin these substances may be beneficial or neutral[35,36]. Many other antigens are also chronically generated, such as cancer-related antigens, cellular debris, modified DNA, and oxidatively modified proteins, which interact with danger-associated molecular patterns (DAMP) to generate and sustain the basal activation of innate immune cells. This is the basis of the inflammaging concept proposed by Franceschi, et al.[37,38]. The most marked changes with aging occur in the adaptive immune system; this is affected phenotypically as well as functionally. The phenotypic changes that were observed pertain to a decrease in the naive T-cell populations and an increase in the memory populations, especially in the $CD8^+$ T-cell compartment. This decrease in the naive T-cells with age is mainly caused by the thymic function diminution[39], but is partly compensated by homeostatic proliferation at the periphery[40,41]. The ultimate consequence of this decrease is loss of diversity of the TCR repertoire, especially in $CD4^+$ naive cells and in $CD8^+$ late-differentiated memory cells. This may result in both increased susceptibility to new infections and decreased response to vaccines as well as poorer memory for previously encountered pathogens. In the meantime, the number of exhausted/terminally differentiated (the so-called senescent) $CD28^-$ T-cells increases, mainly in the $CD8^+$ T-cell subpopulation. The cause of this shift is likely the chronic antigenic stimulation originating either from micro-organismal sources or from internal altered tissue and molecular debris[42]. The most accepted antigenic stimulation in this context is the cytomegalovirus (CMV), which has a tendency to reactivate when the immune surveillance decreases[43]. These observations lead to the determination of the immune risk phenotype (IRP) following the Swedish Octo and Nona studies and were linked to higher mortality during the follow-up period[44,45].

It is of note that there are not only phenotypical changes, but also functional changes either at the level of the T-cell subpopulations or in individual T-cells with aging. There are profound changes in the $CD4^+$ T-cell subpopulations in that the number of T-helper type 2 cells (Th2) and T regulatory cells (T_{reg}) increases with aging. This leads to a further decrease in the adequate adaptive immune response towards new antigens, also altering the memory response. Besides these changes, there are intrinsic alterations in T-cells resulting in altered activation. The membranes of these cells become more viscous because of the increase in cholesterol content with aging[46,47]. These alterations lead to changes in the signaling abilities of different surface receptors including TCR/CD3 complex, cytokine, and co-stimulatory receptors[25,48,49]. Age-associated differences in signaling can be found at almost all stages of the intracellular pathways, but the most important are at the early stages, involving the Src tyrosine kinases (e.g. Lck) and the protein tyrosine phosphatases (e.g. Src homology region 2 domain-containing phosphatase-1, SHP-1)[50]. Our recent data suggest that modulating protein tyrosine phosphatases may increase T-cell responsiveness in the elderly, as was also described by other groups[51].

Together, these changes in the innate and adaptive immunity favor the development of chronic, low-grade (subclinical) inflammatory process (inflammaging) and decrease efficient responses to new infections, cancer, and endogenous tissue injuries, as well as compromising immunity to some previously encountered pathogens[37,52].

Inflammaging

Inflammaging as described above is a state associated with increased proinflammatory mediators, which develops gradually through continuous antigenic stimulation in aged subjects[42,43]. This antigenic stimulation can be provided either by pathogens such as CMV and herpes simplex virus-1, or by cellular and molecular debris arising from transformations caused by reactive oxygen species (ROS), by the Maillard reaction (e.g. advanced glycation end products), by nitrosylation and cancer cells. These constantly generated antigens already stimulate at the resting state both innate and adaptive immunity, resulting in low-grade inflammation[26].

The most common chronic diseases associated with aging and representing an important threat for survival are related to a low-grade inflammatory process. Thus, aging is the most important risk factor for these chronic diseases because of immunosenescence associated with the inflammaging[3,4,54-57]. The most

important of these diseases are atherosclerosis, obesity, diabetes, and neurodegenerative diseases. In each of these diseases, the trigger DAMP interacting with pattern recognition receptors is different as, for atherosclerosis, the oxidized lipoproteins may be the initiator triggers, while in diabetes type 2 these can be the glycated end products, and in neurodegenerative processes these can be the viral products or misfolded proteins. Together, these facts underline the importance of immunosenescence-related inflammaging as a major factor to influence, via these deadly chronic diseases, the longevity of humans[7]. These data further suggest that immune system alterations with aging will be important for determining longevity. Thus, the question arises whether the effects of the altered immune response with aging occur only via disease susceptibility, or if there is another independent mechanism by which altered immunity influences human aging.

In this context, we should also mention that more and more there is a complementary concept stating that the origin of the pro-inflammatory cytokines may be the senescent cells acquiring a senescence-associated secretory phenotype (SASP)[57,58]. These cells seem to accumulate during the aging process and secrete pro-inflammatory cytokines and concomitantly they cannot be eliminated by the immune system when they arise[59]. This cell senescence also in essence is a beneficial process to avoid the development of cancer, but can become dysregulated and contribute to the phenomenon of inflammaging. We have further drawn attention to the fact that pro-inflammatory cytokines alone cannot explain the inflammaging, as this is a very complex process with various interactions between the pro- and anti-inflammatory molecules and the innate immune system[60]. Increased interleukin (IL)-4 production and increased numbers of suppressor cells, such as T_{regs} or myeloid-derived suppressor cells, also exemplify this anti-inflammatory reactive phenomenon, which is most probably not efficient enough in the face of the sustained inflammatory processes with aging[61-64]. Alternatively, it can be too efficient to suppress specific responses, but not efficient enough against sustained, non-specific and multiple-origin, low-level, pro-inflammatory "storm".

Recently, the trained innate memory concept was described[65]. This signifies that innate immune cells may remember the insult far beyond the presence of the insult in the environment. Concomitantly, it became also apparent that an epigenetic memory exists, which can perhaps also maintain the inflammatory state without the original stimulus[66,67]. Finally, the long-lasting pro-inflammatory milieu could also contribute to maintenance of the supranormal immune reaction by self-maintaining it, creating a vicious cycle. It seems that this pro-inflammatory phenotype can at least be detected up to three months after the original stimulation, but presently there are no data for longer follow-up[68].

Further studies on the molecular basis of this long-term memory demonstrated that the trained monocytes/macrophages were characterized by a global increase in epigenetic regulation by H3K4me3 on the promoter region of genes involved in immune signaling and metabolism[69,70]. More recently, the metabolic basis of the trained immunity was uncovered[71]. Thus, the various stimuli for trained immunity include microbes, nutrients, and other stimulating agents that are able to induce a metabolic shift from oxidative phosphorylation to aerobic glycolysis (the Warburg effect). This happens via the activation of mammalian target of rapamycin (mTOR) and its effector, hypoxia-inducible factor (HIF)-1α. So, the mTOR- and HIF-1α-induced metabolic shift play a major role in epigenetic reprogramming of monocytes, leading to the trained innate immunity phenotype of macrophages[72]. Summarizing, we can hypothesize that the trained status of innate immune cells via epigenetic memory presents a persisting pro-inflammatory phenotype maintained by the age-related constant challenges, resulting in maintenance of the functioning of the immune system and also contributing to the appearance of various age-related, chronic inflammatory diseases.

Neurodegenerative diseases

Dementia is highly prevalent among the elderly and increases with age. The question naturally arising in this context is whether low-grade inflammation related to immunosenescence contributes to the development of Alzheimer's disease (AD) by altering the adaptive immune response and favoring an inflammatory status, or whether these changes are only epiphenomena. Recent studies concluded that brain inflammation mediated by microglia is ongoing during the whole neurodegenerative process[73-76]. This has

different intensity in the prodromal mild cognitive impairment stage compared to clinically full-blown AD. Microglia are continuously secreting pro-inflammatory cytokines, while their capacity to phagocytose amyloid beta peptide (Aβ) is seriously compromised as the disease progresses[76]. The production of inflammatory cytokines originating from the innate immune response is stimulated by the increased production of aggregating Aβ[73,77]. These cytokines could have a dual role, since they can be protective by increasing the elimination of Aβ, or they can cooperate as co-stimuli during chronic Aβ stimulation, thereby enhancing a pro-apoptotic effect. Moreover, Aβ primes T-cells, eliciting the appearance of autoreactive T-cells, which can participate either directly or by the production of interferon-γ (IFN-γ) in the destruction of neurons and formation of plaques. It was recently shown that dramatic alterations in naive and memory subsets of $CD4^+$ T-cells occur in patients with mild AD, with greatly decreased percentages of naive cells, elevated memory cells, and increased proportions of $CD4^+$ as well as $CD8^+$ cells lacking the important co-stimulatory receptor CD28. These data provide evidence for more highly differentiated $CD4^+$ as well as $CD8^+$ T-cells in AD patients, consistent with an adaptive immune system undergoing persistent antigenic challenge and possibly manifesting dysregulation as a result, and as such contributing to the pathogenesis of AD[78]. Together, the role of neuroinflammation in AD pathogenesis gains more and more support from experimental data supporting the systemic disease nature of AD and the vicious cycle existing between the periphery and the brain by the re-circulation of the inflammatory cells such as macrophages[73]. Thus, alterations of the immune system contributing to low-grade inflammation with aging might contribute to the development of AD, but the exact role played is still under intense scrutiny.

Diabetes type 2

Diabetes is one of the most common diseases of the elderly, with almost 10% suffering overtly and probably another 10% unrecognized. Diabetes type 2 (T2DM) alone or associated with metabolic syndrome is related to chronic inflammation[52,54,79,80], which in turn is related to the pro-inflammatory activity of the adipose tissue, leading to some degree of insulin resistance and decreased insulin production by pancreatic Langerhans islet cells. It has also been shown that some inflammatory markers, such as F2 isoprostane in urine, IL-6, tumor necrosis factor (TNF), and C-reactive protein (CRP), are increased in T2DM[53]. It has been suggested that the increase of these parameters is associated with increased oxidative stress[81]. T2DM is often associated with complications at the levels of arteries, eyes, and kidneys[82]. These complications are further associated with an inflammatory process exaggerated by the presence of advanced glycation end products. Thus, diabetes is itself an inflammatory disease and, combined with other alterations and with immunosenescence, further alters the immune response.

Atherosclerosis

Atherosclerosis is a typical inflammatory disease and is the pathological basis of cardiovascular diseases, which are extremely frequent in the elderly[83]. This inflammatory disease may be initiated by certain auto-antigens, such as possibly Hsp65, modified low-density lipoproteins, or by infectious agents such as Chlamydia pneumoniae[84]. These agents stimulate cellular immune infiltration of the intima of the arterial wall. The most abundant infiltrating cells are $CD4^+$ T-cells, which bear activation markers[85]. This leads to the secretion of IFN-γ, which in turn stimulates innate immune responses and creates a vicious cycle, leading to clinical events such as acute coronary syndrome with rupture of the plaque[83]. Moreover, a link between the IRP-related $CD8^+CD28^-$ T-cell population and coronary artery disease was demonstrated independently of any CMV infection[86]. This latter finding further suggests that T-cell subset changes during immunosenescence may contribute to the development and progress of atherosclerosis. These cells are now considered as senescent T-cells with SASP[58,59]. Recent work concerning the mechanisms of atherosclerosis also found a persistent pro-inflammatory behavior in monocytes/macrophages with disease progression. In this disease it was found that the inflammatory nature of the peripheral monocytes might predict future cardiovascular events[66]. It is of note that atherosclerosis develops as a life-long process, which starts at a young age and only manifests itself clinically at more advanced age. Together, it seems clear that alterations of the immune system with aging contribute to the development of clinically manifested atherosclerosis such as coronary heart disease.

Chronic heart failure

Chronic heart failure (CHF) affects not only the cardiovascular system, but also neuroendocrine, renal, and immune systems; CHF is associated with a state of chronic inflammation[87]. TNF, IL-6, and IL-1β in the myocardium and peripheral tissues have been shown to play an important role in the pathogenesis and progression of myocardial dysfunction[88]. Indeed, plasma levels of these pro-inflammatory cytokines predict short- and long-term survival in patients with CHF. Furthermore, in hearts of patients with CHF, the accumulation of these TLR-4-regulated pro-inflammatory cytokines and expression of TLR-4 receptor itself have been reported to be increased[89]. Recently, the expression of TLR-4 and TLR-2 was measured on monocytes from CHF patients. Monocyte TLR-4 expression was found to be increased in patients with CHF, and fluvastatin was able to inhibit the excessive innate immune response via inhibition of monocyte TLR signaling[90]. Such increased TLR-4 expression could be a link to the increased proinflammatory cytokines found in CHF. Similar alterations were observed in the TLR system of leukocytes with aging.

GEROSCIENCE

As mentioned earlier, the most important risk factor for all these diseases is aging. Thus, diseases seriously limit not only life expectancy, but also the newly used concept, healthspan. This makes reference to the part of the lifespan that is spent in health and adequate functioning[5-7]. The aim of geroscience is to understand how aging enables chronic diseases, and seeks to develop novel multi-disease preventive and therapeutic approaches. This approach is much larger than what was discussed earlier, as inflammation is only one of the seven pillars of aging, which includes macromolecular damage, epigenetics, adaptation to stress, proteostasis, stem cell and regeneration and metabolism. These putative causes emerged from animal studies and served as a base for intervention studies, which increased longevity and healthspan; however, the basic understanding of the aging process should be the target of intensive basic research. The challenge is to transfer all this knowledge experimentally and therapeutically to humans, aiming to slow the aging process to prolong human healthspan and consequently decrease the appearance of age-related chronic diseases. However, we can envisage that this will be the future for humans with the collaboration of multidisciplinary teams to prevent and avoid the disastrous effects of chronic diseases individually. Thus, targeting aging may allow early interventions and damage avoidance, maintaining a fully functional life and healthspan.

CONCLUSIONS

Aging is a very complex biological and physiological process. Aging results from genetic, epigenetic, and environmental events interacting throughout life. One corollary of aging is that it is the most important risk factor for age-related chronic diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, and diabetes. It was shown that the most common underlying physiopathological process of all these diseases is inflammation. It was also stated that aging is accompanied by changes in the immune system called immunosenescence, characterized by multiple alterations in the phenotypes and functions of the innate and adaptive immune cells. One important characteristic of this immunosenescence is a low-grade inflammation called inflammaging. There is overwhelming evidence that this inflammaging contributes, if not being the origin of, most age-related chronic diseases. The etiology of this inflammaging is still largely unknown, but certainly the disequilibrium between the relatively functioning innate and the more altered adaptive immune system is a contributing factor. The presence of the senescence-associated secretory phenotype is another contributor, and finally, the presence of endogenous damaged macromolecules and cells as well as the leakage of the gut microbiota also participate. Certainly inflammaging is not the only cause of these age-related chronic diseases, but it may represent a sort of common pathway. These chronic diseases shorten the lifespan and also the healthspan. Recently, new efforts of geroscience aimed to more deeply uncover the biological aging process as observed in animal models to apply to humans, and to find pathway targets for slowing down the aging process, leading to a more healthy and functional lifespan by delaying the emergence of these chronic age-related diseases. Among the interventions, those targeting the immunosenescence by thymic replacement, effective vaccine against CMV, targeted anti-inflammatory interventions, or those

targeting senescent cells or the inflammasome have shown promise, but presently mainly in animal models. The way will be very long to get these interventions real in humans.

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Rev Inves Clin. 2016;68:92-8 ORIGINAL ARTICLE

Predictive Value of Frailty Indices for Adverse Outcomes in Older Adults

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ABSTRACT

Background: There are two widely used tools to classify frailty in older adults: the frailty phenotype and the frailty index. Both have been validated for prediction of adverse outcomes. **Objective:** To assess the ability of different frailty indices to predict a number of adverse outcomes (falls, disability, and mortality) by adding deficits in a fixed sequence (with the first five deficits as in the frailty phenotype: weakness, weight loss, slowness, exhaustion and low physical activity) or randomly. **Methods:** This is an analysis of the Costa-Rican Longevity and Healthy Aging Study in which ≥ 60-year-old adults were included and followed up for four years. Frailty indices were constructed, including the frailty phenotype components in the first five indices followed by the random addition of other deficits and estimating for each one the odds ratios for falls and disability and hazard ratios for mortality, adjusted for age and sex. **Results:** We included 2,708 adults; mean age was 76.31 years, 54.28% were women. Indices with the highest number of deficits had the highest estimates for each adverse outcome, independent of the deficit. **Conclusion:** The higher the number of deficits in an index, the higher the estimates for adverse outcomes, independent of the type of deficit added. (REV INVES CLIN. 2016;68:92-8)

Key words: Frail elder adult. Aged. Accidental falls. Disability. Aging epidemiology.

INTRODUCTION

The way individuals age has been the main concern of geriatric medicine in recent years, focusing in particular on the identification of those in whom aging could be considered "pathologic" and are at a higher risk of adverse outcomes (e.g., falls, disability, institutionalization, mortality)[1,2]. Frailty is defined as a loss of the ability to face stressors (internal and external) leading to a longer recovery or transition to a worse health status (e.g., from independence to dependence)[3,4]. Currently, there are a number of scales for the classification of frailty in older adults, with different sets of items and scoring systems and mainly validated by their ability to predict adverse outcomes[5,6]. In fact, there is a substantial heterogeneity within and between tools[7,8], not only in the items they include but also in the scoring system (e.g., some items have higher weights than others)[9-12]. A recent systematic review showed how this heterogeneity results in a wide range of prevalence between studies[13].

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In this context, there is still no agreement on which is the best tool to measure frailty[1,4].

Two main tools to consider an older adult as frail are currently in use in clinical and research settings: the frailty index and the frailty phenotype. The frailty index quantifies a predetermined set of deficits for a given population, with the particular feature of having an arithmetic model base (e.g., arithmetical accumulation of deficits)[14-16]. It has been widely validated in different settings for predicting adverse outcomes[3,7,8]. On the other hand, the frailty phenotype relies on the measurement of five items: gait speed, handgrip strength, physical activity, exhaustion, and weight loss. An older adult that has at least three of the five items is considered to be frail[4]. Different studies also validated this index to predict adverse outcomes.

Our hypothesis is that the items on the frailty phenotype could be taken as deficits in a frailty index; however, when doing this, the higher the number of deficits included in the frailty index will have the highest risk estimates of adverse outcomes, independent of which deficit is added. Therefore, the aim of this study is to assess the ability of different frailty indices to predict a number of adverse outcomes (falls, disability, and mortality) by adding deficits in a fixed sequence (with the first five deficits as in the frailty phenotype: weakness, weight loss, slowness, exhaustion, and low physical activity) or randomly.

MATERIAL AND METHODS

Design and setting

This is a secondary longitudinal analysis of the Costa-Rican Longevity and Healthy Aging Study (CRELES). It is a publicly available dataset of Costa Rican older adults born in or before 1946; it included a representative sample (stratified two-stage probabilistic) of ≥ 60-year-old adults from Costa Rica. A full description of sampling methods and objectives may be found elsewhere[17]. Briefly, this study has three waves in which face-to-face interviews are conducted by trained and standardized staff at the homes of older adults, including in-depth data on demographics, current activities, health-related issues, social support, healthcare use, financial status, functionality, cognitive status, anthropometry, and blood sampling. For the purpose of this report, data from the first (2005) and third (2009) waves were used. Both interviews assessed the same information, with the exception of mortality data in 2009, including a next-of-kin interview for the deceased participants.

Adverse outcomes (dependent variables)

We selected three adverse outcomes to test the predictive ability of the frailty indices: mortality, disability, and falls. As previously mentioned, survival status was obtained from next of kin, and the date of death was recorded to estimate the time (days) to death in the survival analysis (see below). Falls were assessed with the question: "Have you fallen down in the last two years?" If the answer was "yes", the outcome was present. According to the World Report on Disability by the WHO, disability was defined as the "umbrella term for impairments, activity limitations, and participation restrictions". It was operationalized as having incident difficulty in any activity of daily living (from a list of six activities: walking in a room, bathing, eating, moving in and out from bed, toileting, dressing) present in the third wave and absent in the first wave.

Frailty index

As stated previously, 38 deficits were included in the frailty indices: exhaustion, weight loss, low physical activity, slowness, weakness, cognitive decline, spirometry, calf circumference, endurance, reaching test, number of persons living in the same household, self-rated health, hypertension, hypercholesterolemia, diabetes mellitus, cancer, lung disease, heart attack, heart failure, stroke, articular disease, osteoporosis, bone fracture, self-rated vision, self-rated hearing, edentulous, life satisfaction, swelling feet, dizziness, urinary and fecal incontinence, locus of control, self-rated financial status, exercise, recent accident, childhood poverty, self-rated health in childhood, number of hospital days in the last year, and number of currently used drugs. Complete description and scoring of the variables is shown in table 1. Scoring of individual items was rescaled to 0 (absent deficit) or 1 (present deficit), with some items having intermediate scores (e.g., 0.25-0.5-0.75-1). Each item score was added and then divided by 38 to have an overall index score also from 0 (no deficit present) to 1 (all deficits present).

To test our hypothesis, a total of 190 (5 × 38) frailty indices were integrated to be used as independent variables in the regression models and estimate increasing

Table 1. Description of the deficits included in the Frailty Index

Deficit	Definition	Descriptive statistics, n (%)
Exhaustion, n (%)	In the last 12 months, have you had severe fatigue or exhaustion?	1,106 (40.92)
Weight loss, n (%)	In the last 6 months, have you lost 5 or more kilograms unintentionally?	273 (10.98)
Low physical activity, n (%)	Lowest quintile of hours of physical activity of its group by sex	714 (26.37)
Slowness, n (%)	Lowest quintile of gait speed of its group by sex and height	566 (20.9)
Weakness, n (%)	Lowest quintile of handgrip strength of its group by sex and body mass index quartile	561 (20.72)
Cognitive decline, n (%)	Answering less than 75% of the items correctly from a modified version of the MMSE	557 (20.17)
Spirometry in l/min, mean (SD)		202.84 (133.4)
Calf circumference in centimeters, mean (SD)		32.54 (4.23)
Endurance in seconds, mean (SD)	Time to stand up five times from a chair	13.59 (4.72)
Reaching in centimeters, mean (SD)	From a standing position, reach for a pencil on the floor	1.9 (2.53)
Number of persons living with the older adult, median (IQR)	How many persons live in this household?	2 (0-20)
Fair or poor self-rated health, n (%)	How would you rate your health today?	1,366 (50.44)
Hypertension, n (%)	Has a physician ever told you that you have high blood pressure?	1,329 (49.3)
Hypercholesterolemia, n (%)	Has a physician ever told you that you have high cholesterol levels?	937 (35.12)
Diabetes mellitus, n (%)	Has a physician ever told you that you have diabetes (high blood sugar levels)?	523 (19.4)
Cancer, n (%)	Has a physician ever told you that you had cancer or a tumor, excluding small skin tumors?	183 (6.8)
Lung diseases, n (%)	Has a physician ever told you that you have any lung disease such as emphysema, chronic bronchitis, tuberculosis, or asthma?	486 (18.09)
Heart attack, n (%)	Has a physician ever told you that you had a heart attack or infarction?	146 (5.41)
Heart failure, n (%)	Has a physician ever told you that you have heart failure?	371 (13.81)
Stroke, n (%)	Has a physician ever told you that you had a stroke?	146 (5.41)
Articular diseases, n (%)	Has a physician ever told you that you had any articular disease?	446 (16.68)
Osteoporosis, n (%)	Has a physician ever told you that you have osteoporosis?	256 (9.63)
Bone fracture, n (%)	Have you had a bone fracture after your 60th birthday?	462 (17.1)
Self-rated vision, median (IQR)	Rate how good is your vision from one to seven (seven is better)	5.5 (1-7)
Self-rated hearing, median (IQR)	Rate how good is your hearing ability from one to seven (seven is better)	6 (1-7)
Edentulous, n (%)	More than half of the teeth missing	2,349 (86.74)
Not satisfied with life, n (%)	In general, how do you feel with your life?	206 (10.11)
Swelling feet, n (%)	In the last 12 months, have you had swelling feet?	851 (31.45)
Dizziness, n (%)	In the last 12 months, have you had dizziness?	1,034 (38.32)
Urinary incontinence, n (%)	In the last 12 months, have you had involuntary urinary loss?	544 (20.13)
Fecal incontinence, n (%)	In the last 12 months, have you had involuntary fecal loss?	123 (4.55)
Locus of control score, median (IQR)	Set of seven questions exploring health-related locus of control	22 (0-32)
Low self-rated financial status, n (%)	How would you rate your current financial status?	1,643 (60.9)
Not exercised, n (%)	In the last 12 months, have you regularly exercised or done moderate physical activity such as running, biking, or hard work at least 3 days a week?	2,054 (75.91)
Recent accident, n (%)	In the last 10 years, have you suffered from any car accident?	133 (4.82)
Childhood poverty, n (%)	During the first 15 years of your childhood, did your family have economic trouble that prevented you from eating, dressing, or receiving healthcare appropriately?	1,160 (42)
Fair or poor childhood self-rated health, n (%)	How was your health most of the time during your childhood?	173 (6.26)
Hospital days, median (IQR)	How many days were you hospitalized in the last 12 months?	0 (0-200
Drugs, median (IQR)	Number of medications being taken	3 (0-17)

MMSE: Mini-Mental State Examination; SD: standard deviation; IQR: interquartile range.

hazard ratios (HR) for mortality or odds ratio (OR) for falls and disability. The first five indices corresponded to the items of the frailty phenotype, added one by one as follows: weakness, slowness, weight loss, exhaustion, and low physical activity. In the rest of the indices, the variables were added randomly using four different lists of random numbers. For example, for the re-scaled frailty phenotype, the frailty index (FI) was determined as follows: FI = weakness + slowness + weight loss + exhaustion + low physical activity/5; then, the rest of the deficits was randomly added. This procedure was repeated four times; however, as stated previously, only the first five deficits were constant, and from there on, deficits were randomly added.

Statistical analysis

We used descriptive statistics to analyze the variables included in the frailty index, as well as age, sex, and adverse outcomes (falls, disability, and mortality). Each adverse outcome was sequentially used as the dependent variable in logistic multiple regression models for falls and disability, and Cox regression models for mortality. Effects estimates and their 95% confidence intervals (CI) were determined for each adverse outcome and for each of the 190 indices; all models were adjusted for sex and age. Estimates were plotted against the number of deficits for each of the 190 indices and for each adverse outcome. Pearson correlation between the estimates and the frailty indices was also calculated. A statistical significance < 0.05 was considered significant. All analyses were made using STATA 14, and plots were done in Excel 2016.

Ethical considerations

The CRELES was approved by the Ethical Science Committee of the University of Costa Rica (VI-763-CEC-23-04), research project number 828-A2-825. All subjects signed informed consent, and all procedures of the study are according to the last version of Helsinki declaration. In addition, the secondary analysis of this report was registered in the National Institute of Geriatrics of Mexico.

RESULTS

There were 2,708 older adults included; mean age was 76.31 years (± standard deviation [SD] 10.19) and 54.28% were women (n = 1,470). Participants lost to follow-up (n = 419, 15.17%) were significantly younger (73.88 vs. 76.69 years; p < 0.001), were not different in distribution by sex, and had a significantly lower frailty index score (0.243 vs. 0.26; p = 0.004). During follow-up, 531 (19.6%) participants died, 816 (44.32%) had at least one fall, and incident disability was present in 997 (54.1%).

The mean score of the complete frailty index (38 items) was 0.257 (± SD 0.08), with the lowest score being 0.054 and the highest, 0.579. The median was 0.254, the 25th percentile was 0.2, and the 75th percentile, 0.31. The deficit with the highest proportion was having more than half of the teeth missing (edentulous) (n = 2,349, 86.74%), and the lowest for fecal incontinence 4.55% (n = 123). The rest of the deficits are described in table 1.

Regarding mortality, the lowest adjusted HR was found with the lowest number of deficits, independent of the initial deficit; the same occurred with the highest HR, which ranged from 1.01 with one deficit, to 49.17 with all deficits present. The correlation for all the scores was positive and significant, ranging from 0.89 to 0.977 (Fig. 1).

For falls, the lowest OR (1.11) resulted when only one deficit was added to the score, while the OR was 74.12 when all the deficits were present. The correlation for all the scores was positive and significant, ranging from 0.935 to 0.978 (Fig. 2).

Finally, incident or worsening disability also had the lowest estimates when only one deficit was present (OR 1.28) and the highest when all the deficits were present (11.78). The correlation for all the scores was positive and significant, ranging from 0.878 to 0.967 (Fig. 3).

DISCUSSION

According to our results, the deficit-accumulation frailty index seems to be valid in Costa Rican older adults, and when the elements of the frailty phenotype were taken as deficits, there was no difference in risk estimate of adverse outcomes when compared to randomly assembled frailty indices: the higher the number of deficits, the higher the estimates of risk[19]. It has been

Figure 1. Increasing hazard ratios for mortality adjusted for age and sex by adding deficits to the frailty index, either with the first five deficits fixed, as in the frailty phenotype, or in a random sequence.

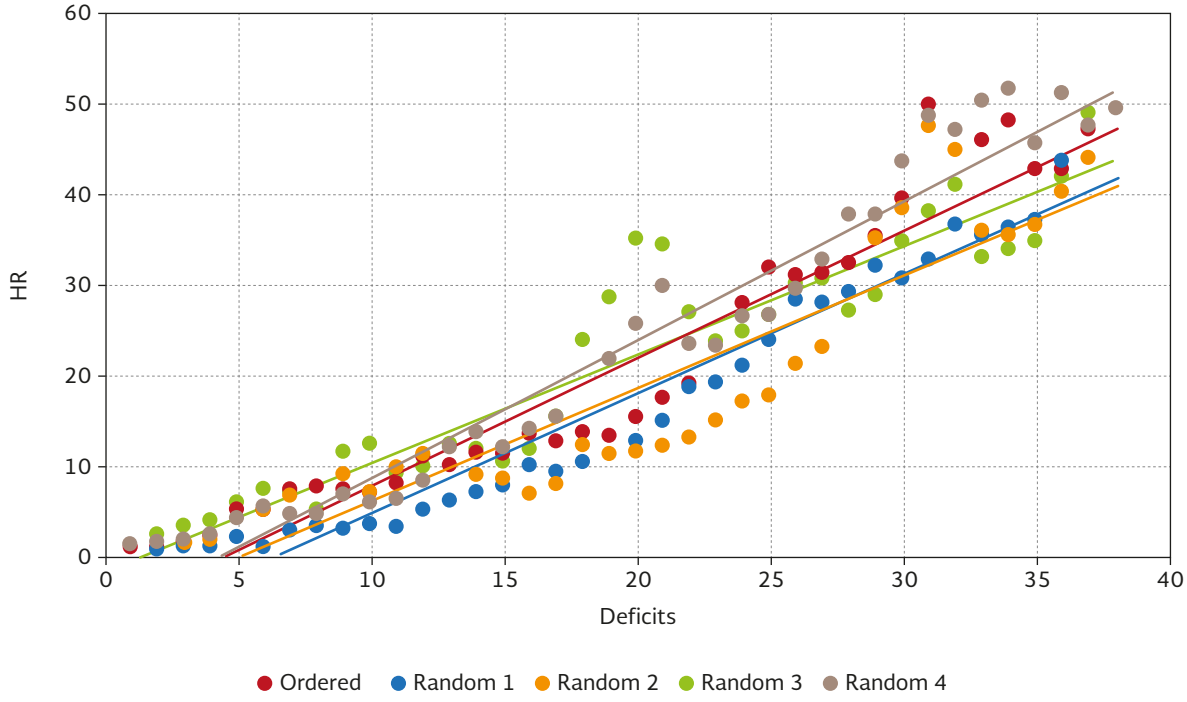


Figure 2. Increasing odds ratio for falls adjusted for age and sex by adding deficits to the frailty index, either arranged as in the frailty phenotype or randomly.

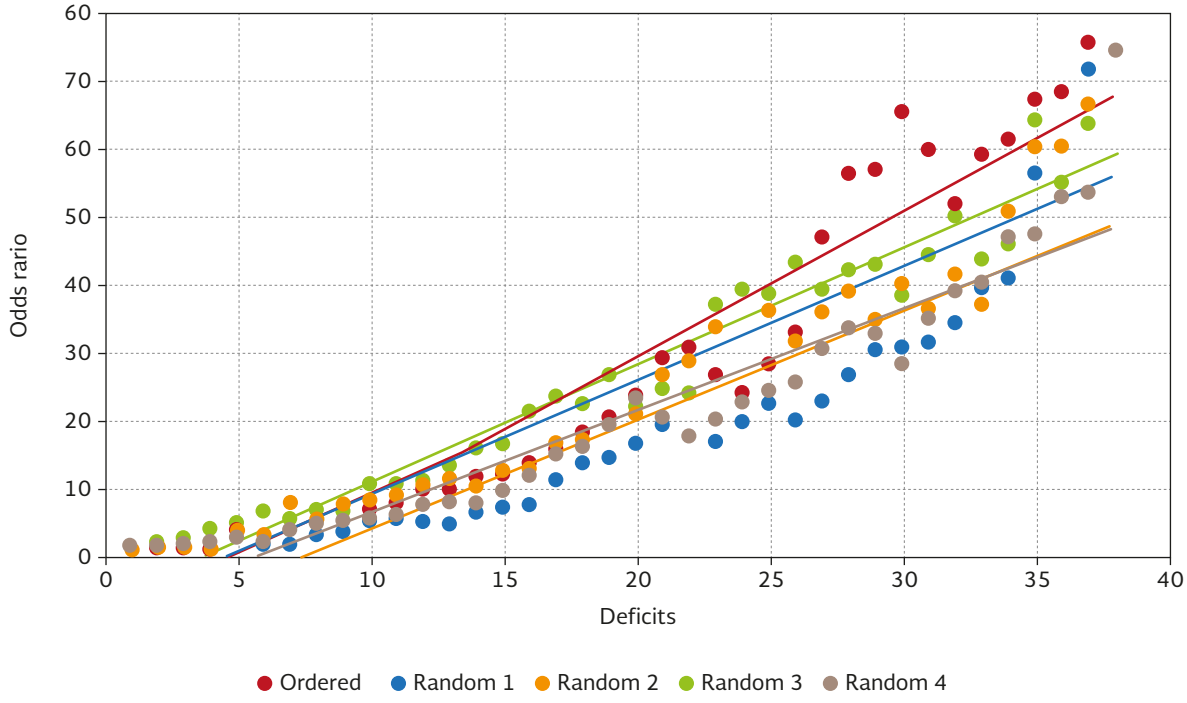
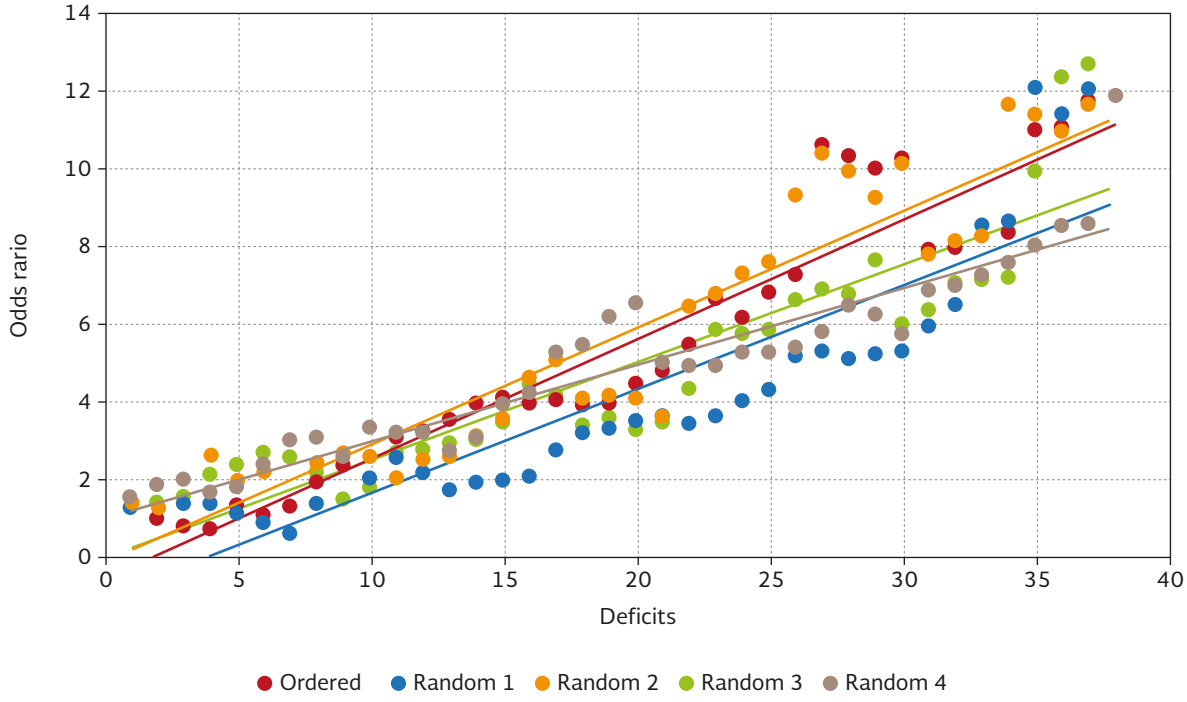


Figure 3. Increasing odds ratio for disability adjusted for age and sex by adding deficits to the frailty index, either arranged as in the frailty phenotype or randomly.



demonstrated that there is an exponential relationship between the number of deficits and death, independent of how the deficits are added[20], and this was also our finding. This may be the same reason why, when adding other items/dominions to the frailty phenotype or other instruments, the estimates of risk of death (or other adverse outcomes) are higher than when considering the phenotype only[21]. The incremental risk of death as the number of deficits increased was also seen in regards to falls and disability, where the OR increased as more deficits were added to the index, resulting in high correlations (> 0.98). Fang, et al. had a similar finding regarding falls and the frailty index, although in their work they did not report data on the linear association between risk of falling and the number of deficits[22]. On the other hand, the frailty index has shown to be predictive of disability in older adults, although an incremental relationship between the number of deficits and the risk has not been described previously[23].

Even though the frailty index and the phenotype may be used for different purposes, our results show that they may not be so unlike, and the integration of both (and any other) into one single tool may be a robust and definitive way of measuring frailty[24,25]. Another feature of the index is its flexibility so that it may be used in almost any setting, including intensive care units, as recently reported[26]. Bearing in mind that the performance of the index (or any other tool) may improve by increasing the number of deficits tested could aid in generalizing and comparing results of multiple studies, considering the number of items included[18].

Although far from being perfect, this index proved useful in this study to solve at least two problems when measuring frailty. The first one is by reducing the weight of a single parameter in its contribution to the overall score of the frailty index. The second is the possibility of computing the frailty index without a predetermined set of variables, but instead basing it on a sufficient amount of heterogeneous information. This feature is of particular interest in the clinical setting where indices could be easily derived from medical records, as previously shown by Jones, et al.[27].

To our knowledge, this is the second report on validation of the frailty index in Latin American older adults and the first in older adults from Costa Rica. The first report on validation of the index and its ability to predict mortality was on Mexican older adults[28]. In

addition, our results provide new evidence on the application of the deficit-accumulation hypothesis in a wide variety of populations and settings.

The number of participants lost to follow-up is one of the main flaws of our study. However, this number was similar to the number of subjects available until the end of the study. The variables we included in the frailty index are not the "classical" ones; we decided to include factors related with social vulnerability and early life experiences to reflect the multidimensional nature of the index.

Further research should aim to implement different indices and to explore how deficits interact and in which way they can provide useful clinical information to help define a problem and intervene in consequence. Meanwhile, the frailty index seems to have advantages over other tools used for frailty detection.

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Original Articles are scientific reports of the results of original clinical, biomedical and translational research. *Original Articles* are limited to six to eight printed pages in lenght including abstract, illustrations, tables and references. Non-essential information (tables, figures and other type of information) can be submitted as supplementary material, which will be only published on line.

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These reviews are four to six journal pages in length, including illustrations and references. They should cover a *focused* area on the advancing edge of medicine providing a balanced view of current advances that can be understood by clinicians and researchers outside of the specialty of the topic. Although these reviews are usually prepared by invitation from the Editors, authors interested in submitting an article to *Brief Reviews* should submit a proposal by e-mail to the *Editor-in-Chief* or *Deputy Editors*, including an outline of the proposed review and a brief CV that includes their publications.

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The abstract should be short and concise, limited to 200 words and should be presented as a **Structured Abstract** (*Background* –not Introduction–; *Objective*, *Methods*, *Results* and *Conclusions*). Do not cite references in the Abstract. Abbreviations can be used but they should be defined only once and at it first use unless it is a standard unit of measurement.

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State clearly the objectives of the work and provide an adequate background, **avoiding** a detailed literature review or a summary of the results. The full term for which an abbreviation stands should precede its first use in the text, no matter if it has been used in the Abstract.

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Acknowledgments

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