BIOLOGICAL MARKERS USED TO IDENTIFY AND EVALUATE FRAILTY IN ELDERLY PEOPLE

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Abstract. Use of biomarkers as feasible endpoints has been proposed for frailty identification, since they would provide a more accurate detection of frail subjects in early stages, when this syndrome can still be potentially reverted. It is possible that, with laboratory parameters, a functional alteration and its evolution can be detected. This should prevent the development of a manifest disease. Experts agreed that no single biomarker by itself is adequate for the assessment of frailty, suggesting a need for a combination of multiple biomarkers. Currently, there are no standardized tests or biomarkers that can be used to identify frail patients and for this reason, identification of biomarkers for frailty is a major consideration for future studies of this syndrome. Scientists working in the field claim that the special measured values which play a specific role in the context of frailty syndrome are there in relation with anemia, vitamins, hormones and inflammatory proteins/cytokines.
Key words: frailty, biomarkers, anemia, vitamins, hormones, inflammaging

MARKERI BIOLOGICI FOLOSIŢI PENTRU IDENTIFICAREA ŞI EVALUAREA FRAGILITĂŢII LA PERSOANELE VÂRSTNICE

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Rezumat. Pentru identificarea fragilităţii a fost propusă folosirea biomarkerilor ca şi obiective fezabile, dat fiind că aceştia ar putea asigura detecţia cu mai mare acurateţe a subiecţilor fragili încă din stadii timpurii, când acest sindrom este potenţial reversibil. Este posibil ca prin intermediul parametrilor de laborator să putem detecta o alterare funcţională şi evoluţia acesteia, ceea ce poate preveni dezvoltarea unei afecţiuni manifeste. Experţii din domeniu sunt de acord asupra faptului că nu există un singur biomarker adecvat pentru evaluarea fragilităţii, sugerând necesitatea combinării mai multor biomarkeri. În prezent nu există teste standardizate sau biomarkeri care să poată fi folosiţi pentru evaluarea pacienţilor fragili şi pentru acest motiv identificarea biomarkerilor fragilităţii este o provocare majoră pentru studiile viitoare asupra acestui sindrom. Oamenii de ştiinţă care lucrează în domeniu susţin că valorile măsurabile cu rol specific în contextul sindromului fragilităţii sunt în legătură cu anemia, unele vitamine, hormoni şi proteine inflamatorii sau citokine.
Cuvinte cheie: fragilitate, biomarkeri, anemie, vitamine, hormoni, "inflammaging"

INTRODUCTION

Frailty, being multi-factorial in etiology, is a multidimensional clinical age-related syndrome affecting multiple organs. Therefore, many biological, clinical and social factors are involved in the development of frailty [1] and all of these factors interact to produce conditions to develop disability and decrease quality of life. It is characterized by increased

vulnerability to stressors, decline of different physiological systems and cognitive abilities, all of these leading to co-morbidity and loss of independence with diminished ability to perform activities of daily living (ADL). For these reasons, it is associated with increased risk of falls and fractures, institutionalization, hospitalization and increased mortality (Fig.1).

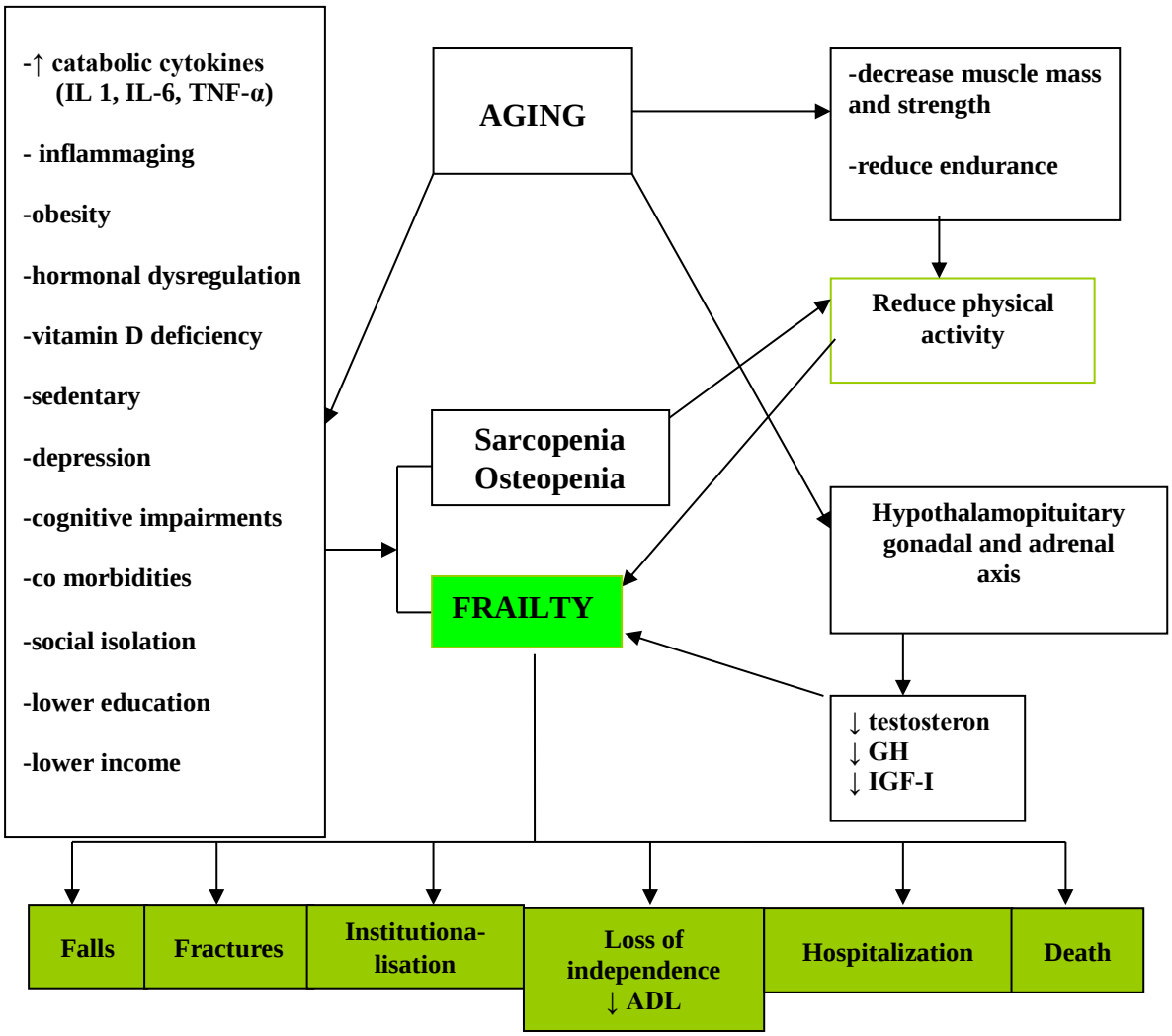

Fig. 1 Diagram representing etiopathogenesis and consequences of frailty

Use of biomarkers as feasible targets has been proposed for frailty identification [2], since they would provide a more accurate detection of frail subjects in early stages, when this syndrome still can be potentially reverted. In this way it is possible that, with laboratory parameters, a functional impairment and its evolution can be detected and this should prevent the development of a manifest disability.

Experts accepted that no single biomarker by itself was able for the assessment of this syndrome, suggesting that it is necessary for a combination of multiple biomarkers. So it was found agreement regarding the necessity to combine biomarkers but no agreement regarding which combination of biomarkers. Until now there are no standardized tests or biomarkers that can be used to identify frail patients and for this reason, identification of biomarkers for frailty is a major consideration for future studies of this syndrome. About this, Ferruci et al. [3] stated: "The factors that contribute to this cyclic metabolic pathway, currently defined as the frailty syndrome,

are still unclear and, therefore, there is still uncertainty on what circulating molecules should be considered as biomarkers of frailty".

The special measured values which play a specific role in the context of frailty syndrome are there in relation with: anemia, vitamins, hormones and inflammatory proteins/cytokines.

Anemia in the context of frailty syndrome

Anemia negatively impacts quality of life and is associated with poorer survival in older adults [4].

Among elderly people with anemia, one third have evidence of folic acid, iron, and/or vitamin B_{12} deficiency, another third have chronic inflammation and/or renal insufficiency, and the remaining third have unexplained anemia, when investigations can not suggest a specific cause (the term "anemia of unknown etiology" - AUE) [5]. One study [6] revealed that erythropoietin (EPO) levels are significantly lower and inappropriately in patients with AUE, suggesting that decreased EPO production may play a key role in the pathogenesis of AUE, being a distinct entity.

EPO is a hormone that plays an important role in the regulation of erythropoiesis, promoting the differentiation and proliferation of the colony-forming unit-erythroid (CFU-E) and other erythroid progenitors. A possible mechanism for the decrease in EPO levels in this elderly population is the presence of a subclinical pro-inflammatory state. Inflammatory cytokines such as IL-1 and TNF alpha are postulated to play a role in development of anemia of chronic disease through inhibition of EPO synthesis.

Iron deficiency is of particular significance, but the most important criterion to make difference between pathologies (chronic inflammation and iron deficiency) is the serum concentration of the ferritin (iron storage protein). This is lowered in anemia with iron deficiency and raised in anemia of chronic diseases.

Another cause of anemia is the deficiency of folic acid and/or vitamin B12, resulting in the impaired DNA metabolism. Therefore hyperchromic, macrocytic anemia develops, which is characterized by an increased mean erythrocyte volume (VEM) with a simultaneously high hemoglobin content of the erythrocytes.

Vitamins in the context of frailty syndrome

The importance of folic acid and B12 vitamin in the development of frailty has already been explained, but another vitamin which is important for the functionality of the elderly is D vitamin, with it's especially role in the regulation of calcium metabolism (stimulation of bone mineralization, absorption of calcium in the intestine, primary role in the maintenance of extra cellular fluid calcium concentration). The association between D vitamin deficiency and bone disease are well recognized, but have been increasing indications that the D vitamin system plays an important role in the metabolism of a wide variety of cells and systems, so as can be seen in Tab. I.

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Tab. I D Vitamin deficiency and associated conditions

Cardiovascular	orthostatic hypotension, aortic dilatation
Respiratory	obstructive sleep apnea, bronchiolitis, bronchiectasis, asthma
Gastrointestinal	chronic hepatitis, liver cirrhosis, pancreatitis
Neurological	myasthenia gravis, multiple sclerosis
Musculoskeletal	osteoporosis,osteoarthritis, rheumatoid arthritis, muscle weakness, falls, fractures
Metabolic	diabetes mellitus, diabetic nephropathy, metabolic syndrome
Cancer	colorectal, lung ovarian, prostate, breast
Skin	systemic lupus erythematosus, eczema, psoriasis

D vitamin deficiency is associated with muscle weakness predominantly of the proximal muscle groups. This leads to slower walking speed, prolonged sit-to-stand time, lower quadriceps strength, and a higher rate of falls [7] which are a major problem in the elderly, leading to significant morbidity, increased mortality and substantial consumption of healthcare resources. Observational findings have been confirmed by interventional studies with daily dosing of D vitamin from 800 to 1000 IU per day associated with a 20– 30% reduction in falls rate and significant improvements in body sway [8].

Osteoporosis causes substantial morbidity and mortality in older people, but whether chronic insufficiency of D vitamin is a reversible determinant of osteoporosis and related risk of fractures is controversial. Observational studies indicate that low plasma levels of 25 OH vitamin are associated with higher risk of fractures and with vascular and non-vascular mortality [9-12], but it is unclear if these associations are causal. Randomized trials assessing the effects on fracture and other health outcomes have generally failed to demonstrate beneficial effects of D vitamin supplementation [13-16], but in a major study in subjects over 65 years old, Trivedi et al. [17] showed that the additional intake of D3 vitamin reduced the fracture rate with 33%.

Hormones in the context of frailty syndrome

The process of aging strongly affects entire endocrine system, because some target tissues become less sensitive to their mechanism of hormonal control or the amount of hormones produced may also change (Tab. II).

Four main groups of hormones are involved in frailty: thyroid hormones; growth hormone/insulin-like growth factor-1 (GH)/IGF-1 and insulin; sexual hormones (testosterone and estradiol); cortisol/dehydroepiandrosterone (DHEA).

Tab. II The association of variation of the hormonal profile in the process of aging with longevity and frailty

	Hormones	**Variations in aging**	**Longevity**	**Frailty**
1	DHEA	↓	Higher levels with predictive factor for long-term longevity	Predict the risk of developing frailty
2	Testosteron	↓	Associated with low longevity in men and women	Prevalence of frailty increases, whereas testosterone decreases, as men age.
3	Estrogens	↓	Long-term replacement therapy is associated with lower all-cause mortality in older women.	In contrast to testosterone, frailty seems to be associated with high estrogen levels in postmenopausal women (especially if are associated to inflammation)
4	GH	↓	Survival to extremely old age - associated with reduced GH and IGF-1 signaling	Associated +/-(possible utility of GH in the treatment of frailty and sarcopenia)
5	fT4/TSH	↑	Inverse correlation suggest a potential role of decreased thyroid function leading to longevity	fT4 within normal range associated with frailty in ♂

Thyroid gland is certainly impacted in elderly, the prevalence of thyroid disorders increasing with age. Subclinical disturbances of thyroid function are more frequent in the elderly and the symptoms are more subtle, often attributed to normal aging. The natural history of subclinical hypothyroidism depends on the presence

or absence of antithyroid peroxidase antibodies (TPOAb). Thus, a quite high rate of reversion of subclinical hypothyroidism to euthyroid status in adults aged at least 65 years with lower baseline TSH levels and TPOAb negativity was observed [18]. In turn, higher TSH level and TPOAb positivity were independently associated with lower chance of reversion to euthyroidism [18]. Moreover, TSH levels ≥ 10 mIU/l were independently associated with progression to overt hypothyroidism [18]. Similar findings, showing that higher baseline TSH levels are associated with progression from subclinical to overt hypothyroidism and that higher TSH level (>8 mIU/l) is a predictive value for development of overt hypothyroidism, were recently reported by Imaizumi et al. [19]. On the other hand, there is strong evidence that thyroid hypofunction may contribute to increased lifespan (see further in the text). Therefore, taking into account all mentioned observations, the replacement therapy with L-thyroxine is not uniformly recommended in elderly people with subclinical hypothyroidism.

DHEA is a steroid precursor of testosterone produced by the adrenal cortex and the biological role of it is not yet well defined. Observational studies have demonstrated that plasma levels of DHEA and DHEAS decline by 80% between 25 and 75 years, and this decrease is greater after 80 years [20], serum DHEAS being a predictive factor for long-term longevity. The pattern of steroid secretion from corticoadrenal gland shows a progressive significant reduction of DHEAS levels, being cortisol nearly unchanged, and consequently with a significant age related increase of cortisol/DHEAS ratio. Therefore, low DHEA levels and a high cortisol/DHEA ratio predict the risk of developing frailty.

Data from the InCHIANTI study [5] suggest that DHEAS levels are related to lower extremity muscle strength and have reported that frail people have lower levels of serum IGF-I and DHEAS and higher levels of IL-6 than non-frail, age-matched individuals.

The physiological pathways leading to frailty are complex, but there is evidence that testosterone may play an important role in aging men.

Testosterone

- promotes erythropoesis
- maintains bone mineral density
- supports muscle function and growth
- anabolic actions
  - -stimulate appetite and food intake
  - -increase protein synthesis
  - -inhibition of adipocyte production
  - -stimulation of cells for muscle repair
  - -enhance amino acid reuse in muscle

Hypogonadal men typically present:

- decreased bone mineral density
- loss of energy
- muscle wasting

Several studies have explored the association between sex hormones and various components of the frailty syndrome. In 2005 Schaap and colleques [21] reported results of a cross-sectional analysis in older men. Total and free testosterone levels were positively associated with grip strength, whereas free testosterone levels were associated with better mobility and were less likely to fall during a follow-up period of 4 years. These findings suggest that testosterone therapy could potentially treat or prevent the development of frailty.

Estrogens may have a central role in the long life expectancy of women. A study

from 2012, Carcaillon et al. [22] found the potential association between estradiol (E2) levels and frailty among older postmenopausal women not taking hormonal therapy. The results demonstrated that E2 levels decreased significantly with age and educational level; whereas they increased with body mass index and high-sensitivity C-reactive protein (hs-CRP). Higher E2 levels were associated with the prevalence of frailty among postmenopausal women younger then 79 years, but not in the oldest group.
The synergism between higher E2 and hs-CRP levels suggests the existence of physiopathological mechanisms connecting inflammation and estrogen to frailty.

Estrogen levels correlates to:

- favorable impact on serum lipid profiles
- high risk for breast and ovarian cancer
- low risk for heart disease, stroke and all cause mortality
- protective effects on cerebral areas known to be involved in age-related cognitive functions and Alzheimer's disease.

Growth hormone (GH), produced in the anterior pituitary gland, is released into the circulation in a pulsatile manner and predominantly stimulates the liver to produce IGF-1. The GH /IGF-1 axis in aging, like deficiencies in sex steroid hormones, appears to be linked with changes in the hypothalamic-pituitary-adrenal axis, affecting muscle and bone physiology. GH levels in men and women fall significantly with age. Also, decline in IGF-1 often occurs with increasing age, and low IGF-1 levels are associated with frailty and an increased risk of death. In this same regard, insulin resistance is also associated with frailty, supporting the contribution of low-functioning insulin/IGF-1 signaling to a prolonged survival, but at the same time, to the presence of frailty.

Inflammation in the context of frailty syndrome

Chronic inflammation has been postulated like a key mechanism involved in frailty, acting either directly or indirectly through its negative influence on other physiological systems [23]. Thus, studies testing the hypothesis that frailty is associated with alterations in the concentration of immune activation markers, in different lymphocyte subpopulations, and pro-inflammatory molecules are becoming more in the last years [24].

Cross-sectional studies reported [25] that compared to robust participants; both frail and pre-frail ones had significantly higher levels of C-reactive protein. Frailty and pre-frailty were associated with higher serum levels of IL-6, and were also significantly associated with elevated number of white blood cells/mmc and fibrinogen levels compared to people who were robust.
The study of Marcos-Perez M et al. published in may 2018 [26] confirmed the involvement of chronic inflammation in frailty in later life; particularly strong associations were obtained in the regression analysis for IL-6 and for sTNF-RII. This last biomarker showed a high accuracy for predicting frailty. Although results from this study revealed limited strength associations between frailty and the lymphocyte subsets, data obtained for the different inflammatory mediators provide additional reinforcement to the widely established hypothesis that inflammaging is involved in the frailty status in older adults.
There is a complex physiological interaction between various anabolic hormones (GH, IGF-I and testosterone), inflammatory cytokines (IL-2, IL-6, TNF-α), biochemical and molecular pathways mediating catabolism of muscle protein, age related muscle loss and consequently to physical frailty.

CONCLUSIONS

I. Frailty becomes more prevalent with increasing age but is not an inevitable consequence of aging
II. Use of biomarkers as feasible endpoints has been proposed for frailty identification, since they would provide a more accurate detection of frail subjects in early stages, when this syndrome can still be potentially reverted
III. Experts agree that there is no single biomarker there is adequate to predict or identify frailty, but it is necessary a combination of multiple biomarkers
IV. Additional research work is needed to identify the specific combination of clinical and laboratory biomarkers that can be used for the diagnosis of frailty
V. It can be stated that laboratory diagnostic, in addition to clinical observation and anthropometric data, represent important components for the clarification of preventive approaches and the causality of the frailty

Conflicts of interest

The authors declare no conflicts of interest.

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