

Biomarkers in sarcopenia: A multifactorial approach



Francesco Curcio ^a, Gaetana Ferro ^a, Claudia Basile ^a, Iliaria Liguori ^a, Paolo Parrella ^a, Flora Pirozzi ^a, David Della-Morte ^{b,c}, Gaetano Gargiulo ^d, Gianluca Testa ^e, Carlo Gabriele Tocchetti ^a, Domenico Bonaduce ^a, Pasquale Abete ^{a,*}

^a Department of Translational Medical Sciences, University of Naples “Federico II”, Naples, Italy

^b Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy

^c IRCCS San Raffaele Pisana, Rome, Italy

^d Division of Internal Medicine, AOU San Giovanni di Dio e Ruggi di Aragona, Salerno, Italy

^e Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy

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ABSTRACT

The slow and continuous loss of muscle mass that progresses with aging is defined as “sarcopenia”. Sarcopenia represents an important public health problem, being closely linked to a condition of frailty and, therefore, of disability. According to the European Working Group on Sarcopenia in Older People, the diagnosis of sarcopenia requires the presence of low muscle mass, along with either low grip strength or low physical performance. However, age-related changes in skeletal muscle can be largely attributed to the complex interactions among factors including alterations of the neuromuscular junction, endocrine system, growth factors, and muscle proteins turnover, behavior-related and disease-related factors. Accordingly, the identification of a single biomarker of sarcopenia is unreliable, due to its “multifactorial” pathogenesis with the involvement of a multitude of pathways. Thus, in order to characterize pathophysiological mechanisms and to make a correct assessment of elderly patient with sarcopenia, a panel of biomarkers of all pathways involved should be assessed.

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1. Biomarkers of sarcopenia: an unmet need

Skeletal muscle annually loses about 0.1–0.5% of its mass starting at the age of 30, with a dramatic acceleration of this process after the age of

65. This phenomenon has been defined as “sarcopenia” and is related to a series of delicate economic and social implications, including hospitalization and death (Melton et al., 2006; Chumlea et al., 2011; Morley et al., 2011; Liu et al., 2014). The term “sarcopenia” was first introduced by Rosenberg in 1989 (Rosenberg, 1989) and, successively, Baumgartner and colleagues proposed an identification method of sarcopenia based on lean mass evaluation by dual-energy X-ray absorptiometry (DEXA) (Baumgartner et al., 1998). In addition, “sarcopenic

* Corresponding author at: Dipartimento di Scienze Mediche Traslazionali, Università di Napoli Federico II, Via S. Pansini, 80131 Naples, Italy.
E-mail address: p.abete@unina.it (P. Abete).

obesity” has been defined as a loss of muscle mass accompanied by an increase in fat mass relative to a fat-free mass, and is a predictor of worse clinical outcomes (Roubenoff, 2000). Finally, it has also been suggested that sarcopenia and osteoporosis could be the extreme results of a common pathway of tissue depletion defining the condition of “osteosarcopenia” (Sjöblom et al., 2013).

According to the operational definition by the European Working Group on Sarcopenia in Older People (EWGSOP), the diagnosis of sarcopenia requires the presence of low muscle mass (estimated by the ratio of appendicular lean mass over the height squared, ≤ 8.0 kg/ht² for men and ≤ 6.0 kg/ht² for women), in the presence of low physical performance (a gait speed < 0.8 m/s and/or a grip strength < 26 – 30 kg for men and < 16 – 20 kg for women) (Cruz-Jentoft et al., 2010). Muscle mass evaluation remains the main problem for the diagnosis of sarcopenia. Although Dual energy X-ray absorptiometry (DEXA) (Binkley et al., 2013) and Bioelectrical Impedance analysis (BIA) (De Rui et al., 2016) are largely utilized for the assessment of skeletal muscle mass, Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) represent the gold standard and the most accurate imaging methods to provide not only an exact measurement of muscle mass, but also important data on its density and fatty infiltration (Goodpaster et al., 2000).

The major problem in the diagnosis of sarcopenia is its multifactorial genesis (Lauretani et al., 2014; Santilli et al., 2014). In fact, the pathophysiology of sarcopenia includes endocrine dysfunctions, inflammatory conditions, and glucose, glycogen, and lipid metabolism alterations (Malafarina et al., 2012; Pedersen and Febbraio, 2012). Moreover, muscle-related cytokines and myokines seem to show auto-, para- and endocrine actions cross-talking between muscle and tissues such as bone, fat, and liver. In addition, a number of factors related to chronic diseases and uncorrected lifestyles (i.e. anorexia, obesity and low physical activity) may determine the development of sarcopenia (Biolo et al., 2014; Sakuma et al., 2015). Despite promising advances in evaluating muscle mass and strength, the multiple mechanisms at the basis of sarcopenia have not been fully characterized, yet; nevertheless, a series of biomarkers may be found in both tissue and blood samples (Kalinkovich and Livshits, 2015). Histology still represents the gold standard for the recognition of the pathophysiological mechanisms of different sarcopenic syndromes; however, biopsy samples are often unavailable for ethical reasons and not agreeable to elderly patients. In

addition, during follow-up of sarcopenic patients, several tissue samples would be needed. Thus, the emerging priority is to identify potential biomarkers for early selection of patients at risk for sarcopenia among those with age-related loss of muscle mass. Here, we aim at defining a pool of blood biomarkers that may help characterize the different mechanisms of sarcopenia in different patients, allowing for a personalized follow-up for the effectiveness of prevention and treatment measures (Barbat-Artigas et al., 2014; Dutt et al., 2015).

2. Sarcopenia biomarkers

Sarcopenia not only includes tissue loss and contractile dysfunction, but also endocrine and metabolic abnormalities, with close interactions with the low-grade age-related systemic inflammation (i.e. “inflammaging”) (Beyer et al., 2012; Ilich et al., 2014) (Fig. 1). Thus, as a matter of fact, the muscle is no longer seen as a simple contractile motor, but as a crossroads of more complex networks, involving a reduction of protein-synthesis and regeneration, with a parallel increase of apoptosis and protein-lysis. Specific biomarkers would be related to clinically assessment, and therefore, would allow to detect the subjects affected or at risk of sarcopenia and to follow up the effectiveness of prevention and treatment measures. Therefore, we could identify biomarkers of sarcopenia according to different pathophysiological mechanisms:

- 2.1. Neuromuscular junction
- 2.2. Endocrine system
- 2.3. Growth factor
- 2.4. Muscle protein turnover
- 2.5. Behavior-mediated pathways
- 2.6. Inflammation-mediated pathways and redox-related factors

Table 1 shows the different pathophysiological pathways with related biomarkers and associated diseases.

2.1. Neuromuscular junctions

One of the most investigated mechanisms involved in the pathogenesis of sarcopenia is the impairment of the neurophysiological functions,

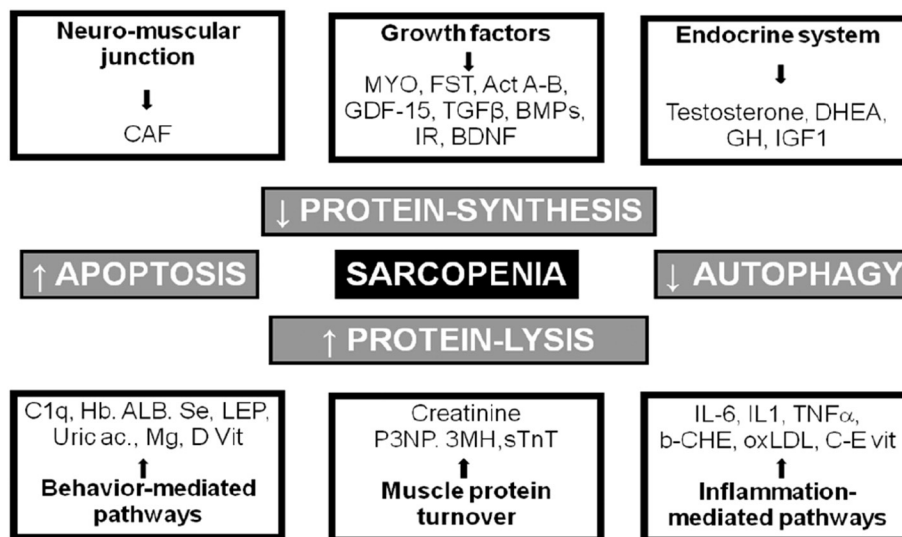


Fig. 1. Pathophysiology mechanisms of sarcopenia and related biomarkers. Legend: CAF = C-terminal agrin fragment; DHEA = dehydroepiandrosterone; GH = growth hormone; IGF-1 = Insulin-like growth factor 1; MYO = Myostatin; FST = Follistatin; Act = Activin; GDF-15 = Growth Differentiation Factor-15; TGFβ = Tumor Growth Factor β; BMPs = Bone morphogenetic proteins; BDNF = Brain-Derived Neurotrophic Factor; ALB = Albumin; Hb = Hemoglobin; P3NP = N-terminal peptide; 3MH = 3-methylhistidine; sTnT = Skeletal muscle-specific isoform of troponin T; IL-6 = Interleukin 6; IL-1 = Interleukin 1; oxLDL = Oxidized low-density lipoprotein; TNFα = tumor Necrosis Factor alpha; b-CHE = Butyrylcholinesterase.

Table 1
Relationship among biomarkers and related mechanisms.

Mechanism	Biomarker	Pathogenesis	Modification	Associated disease	Main reference
Neuromuscular junctions	<i>C-terminal agrin fragment</i>	Impairment of neuromuscular junctions	Increase	Immobilization syndrome, chronic kidney disease	Gonzalez-Freire et al. (2014)
	<i>Myostatin</i>	Muscle growth suppressor	Increase		
	<i>Activins A and B</i>	Muscle growth suppressor	Increase		Chen et al. (2014)
	<i>Follistatin</i>	Muscle growth promoter	Decrease		Lee and McPherron (2001)
Growth factors	<i>Growth differentiation factor-15</i>	Muscle growth suppressor	Increase	Cancer, Cachexia, muscular dystrophy	Bloch et al. (2013)
	<i>Tumor growth factor β</i>	Muscle growth suppressor	Increase		
	<i>Bone morphogenetic proteins</i>	Muscle growth promoter	Decrease		
	<i>Irisin</i>	Muscle growth promoter	Decrease		Sartori et al. (2014)
	<i>Brain-derived neurotrophic factor</i>	Muscle growth promoter	Decrease		Massagué et al. (2005)
	<i>Testosterone</i>	Muscle growth promoter	Decrease	Andropause	Vamvini et al. (2013)
Endocrine system	<i>Dehydroepiandrosterone</i>	Muscle growth promoter	Decrease		Brunelli et al. (2012)
	<i>Growth hormone</i>	Muscle growth promoter	Decrease	Somatopause	Bhasin et al. (2006)
	<i>Insulin-like growth factor 1</i>	Muscle growth promoter	Decrease		Meng et al. (2015)
	<i>Skeletal muscle-specific troponin T</i>	Contractile insufficiency	Increase		Sakuma and Yamaguchi (2012)
Muscle protein turnover	<i>N-terminal type III procollagene</i>	Muscle remodeling	Decrease	Muscular dystrophy	Giovannini et al. (2008)
	<i>3-Methylhistidine</i>	Proteolysis of myofibrils	Increase		Abreu et al. (2014)
	<i>Creatinine</i>	Muscle turnover reduction	Decrease	Immobilization syndrome, Malnutrition	Bhasin et al. (2009)
	<i>Complement protein C1q</i>	Physical inactivity	Increase	Immobilization syndrome	Sheffield-Moore et al. (2013)
	<i>Hemoglobin</i>	Inadequate intake/underproduction	Decrease	Malnutrition, myelodysplastic syndrome	Stimpson et al. (2013)
Behavior-mediated pathways	<i>Albumin</i>	Inadequate intake/underproduction or lack	Decrease	Malnutrition, kidney and/or hepatic chronic disease	Watanabe et al. (2015)
	<i>Selenium</i>	Inadequate intake	Decrease	Malnutrition	Penninx et al. (2004a, 2004b)
	<i>Leptin</i>	Obesity	Increase	Metabolic syndrome	Lauretani et al. (2007)
	<i>Uric acid</i>	Inadequate intake	Decrease		Fuentes et al. (2010)
	<i>Magnesium</i>	Inadequate intake	Decrease	Malnutrition	Macchi et al. (2008)
	<i>Vitamin D</i>	Inadequate intake	Decrease	Osteoporosis	Dominguez et al. (2006)
	<i>Interleukin 6</i>	Inflammation	Increase		Visser et al. (2003)
	<i>Tumor necrosis factor α</i>	Inflammation	Decrease		Sell et al. (2012)
	<i>Interleukin 1</i>	Inflammation	Decrease		Trendelenburg et al. (2012)
	Inflammation-mediated pathways and redox-related factors	<i>Butyryl-cholinesterase</i>	Inflammation	Decrease	Chronic degenerative disease
<i>Oxidized low-density lipoprotein</i>		Pro-oxidant	Increase		Cacciatore et al. (2015)
<i>C-E vitamin</i>		Anti-oxidant	Decrease	Malnutrition	Cesari et al. (2005)
					Semba et al. (2007)

which seem to be associated to a dysfunction of neuromuscular junctions (NMJs) (Gonzalez-Freire et al., 2014) (see Table 1).

NMJs are involved in the transduction of the action potentials of muscle, and their dysfunction could lead to a gradual alteration of this process during exercise, associated with neuromuscular fatigue. Consequently in the elderly this would create an impaired response to physical training, because of the difficulty in accomplishing a durable exercise (Rudolf et al., 2014).

It has been hypothesized that NMJ dysfunction could be due to an increased proteolytic cleavage of agrin (Bütikofer et al., 2011). Agrin is a protein synthesized by motoneurons that seems to activate the receptor tyrosine kinase muscle-specific (MuSK), that stabilizes the acetylcholine receptor (AChR). Neurotrypsin, a protease of synaptic origin, would cleave agrin, producing a C-terminal agrin fragment (CAF, measurable in serum), with a consequent destabilization of AChR (Bolliger et al., 2010; Drey et al., 2013). Supporting this hypothesis, some studies have shown, that CAF circulating levels are much higher in sarcopenic

than in non-sarcopenic subjects (Hettwer et al., 2010; Marzetti et al., 2014a). Interestingly, recent observations highlighted an inverse correlation between circulating levels of CAF and neuromuscular fatigue obtained by the measure of vastus lateralis muscle physical work capacity threshold (Stout et al., 2015) and, in elderly patients, a link between CAF circulating levels and the loss of appendicular lean mass (Drey et al., 2013).

2.2. Endocrine system

Sarcopenia is characterized by a variable decline of several hormones (see Table 1), especially sex hormones (e.g. testosterone and dehydroepiandrosterone (DHEA), growth hormones (e.g. growth hormone (GH) and Insulin-like growth factor 1 (IGF-1)).

It is well known that Testosterone (that decreases by 1% per year since the age of 30 in males) increases muscle protein synthesis (Sakuma and Yamaguchi, 2012). A review of the literature (Bhasin et

al., 2006) concluded that testosterone supplementation reduces the reduction in muscle mass and grip strength. However, the improvement in strength among elderly males has been obtained with high doses of testosterone, and therefore, the potential risks (i.e. sleep apnea, thrombotic complications, and the increased risk of prostate cancer) may outweigh the benefits (Sakuma and Yamaguchi, 2012).

DHEA, secreted by the adrenal cortex, is a major androgen able to regulate muscle growth. The fact that DHEA levels decrease with age suggests that this hormone likely plays an important role in the pathogenesis of sarcopenia. DHEA may induce beneficial age-related effects on body composition and physical performance (Maggio et al., 2013). Unfortunately, despite the close relationship between anabolic hormones, muscle mass and strength, some studies failed to show their relationship with the reduction of strength (Meng et al., 2015).

Growth hormone (GH) is a single-chain peptide produced by the anterior pituitary gland. Its production is modulated by the actions of GH-releasing hormone (GHRH), which stimulates GH secretion, and somatostatin, which inhibits GH secretion. Similarly to testosterone, GH levels decline progressively after the age of 30 at a rate of ~1% per year but, more importantly, daily GH secretion is 5–20-folds lower than that in young adults. The age-dependent decline in GH secretion is secondary to a decrease in GHRH and to an increase in somatostatin secretion. The growth-promoting actions of GH are mediated by circulating or locally produced IGF-1 (Sakuma and Yamaguchi, 2012), that is considered a potent anabolic hormone, well known to stimulate muscle growth and regeneration. It has been demonstrated that systemic IGF-1 administration increases the rate of skeletal muscle functional recovery after injury (Giovannini et al., 2008).

It has also been proposed that the development of sarcopenia may be provoked by thyroid pathologies. However, although women with subclinical hypothyroidism had a higher prevalence of sarcopenia, it was shown that TSH levels were not associated with muscle mass, strength or quality (Moon et al., 2010).

2.3. Growth factors

One of the theories about the onset of sarcopenia refers to an imbalance between muscle cells growth enhancer and suppressor factors, in favor of the latter.

Myostatin (MYO) received great attention among the pathophysiological mechanisms of sarcopenia. This molecule was initially named growth and differentiation factor 8 (GDF-8) in animal models of hypermuscularity (McPherron et al., 1997). Its powerful action as a negative muscle growth regulator is confirmed by the evidence that MYO-knockout mice show abnormal muscle hypertrophy, while MYO overexpression leads to severe atrophy (Lee and McPherron, 2001). MYO belongs to the large TGF β family and is mainly secreted by muscle fibers and, partly, by adipocytes (Raschke and Eckel, 2013).

In consideration of its biological significance, an intense research on the role of MYO in the development of sarcopenia has been carried out, but data are controversial and even discordant. In some studies, elevated serum protein and mRNA levels of MYO were detected in older compared to young subjects (Léger et al., 2008; Yarasheski et al., 2002). In contrast, other studies found a significant elevation in MYO mRNA and protein levels in young compared to older males (Sakuma et al., 2014). Several hypotheses have been postulated to explain this discrepancy. Indeed, MYO levels may not reflect MYO activity, but only its precursor protein, that is regulated by three interacting proteins, i.e. GDF-associated serumprotein-1 (GASP1), follistatin, and follistatin-related gene (FLRG) that may be independently affected by aging. Finally, the expression of MYO during sarcopenia may be detected only in satellite cells, but not throughout the entire muscle fibers (Sakuma et al., 2014).

Activin A and B, more members of the TGF β superfamily, seem to exercise an important action in muscle mass regulation. In some mouse models of cancer-induced cachexia, they were found to be 100-fold

more effective in causing muscular wasting, compared to MYO (Chen et al., 2014).

Follistatin (FST) is considered the main inhibitor of MYO in the process of muscle wasting. MYO is secreted in serum as an inactive form, bound to inhibitory proteins, above all to FST. An overexpression of FST in animal models leads to a significant increase in muscle mass (Lee and McPherron, 2001). The role of FST on muscle growth is not limited exclusively to the modulation of MYO action, but FST is also supposed to regulate more factors involved in myogenesis. The increase of muscle mass in animals that over-expressed FST was greater than the one observed in MYO knock-out animals, and muscle increase was even greater in those which presented both a FST transgene and a deletion of the MYO gene (Lee, 2007). The underlying mechanisms of the increase in FST-induced muscle repair are not fully characterized, but are probably due to FST ability to inhibit MYO, activin A and TGF β , with a consequent enhancement in muscle progenitor cells-supported myogenesis. (Gilson et al., 2009; Lee et al., 2005; Zhu et al., 2011). These observations indicate FST as an interesting clinical tool in the monitoring of muscle damage or muscle atrophy and as a potential sensitive marker of sarcopenia. Finally, the modulation of Activin receptor II (ActRII) B, which regulates MYO signaling, causes muscle atrophy while a blockade of this pathway leads to muscle hypertrophy and increases strength and physical performance both in animals or in humans. In particular, Bimagrumab is a monoclonal antibody that improves muscle strength, mass and function by inhibiting the ActRII (Molfinio et al., 2016).

Importantly, MYO, activins and FST levels may be all measured in serum (Breitbart et al., 2013).

Protein and mRNA levels of Growth Differentiation Factor-15 (GDF-15), another member of the TGF β superfamily, are related to muscle atrophy in patients undergoing cardiac surgery (Bloch et al., 2013). Interestingly, an increased expression of GDF-15 was associated with a reduced expression of some microRNAs involved in the regulation of muscle growth in patients hospitalized in intensive care (Bloch et al., 2015). These findings suggest a direct role of GDF15 as a suppressor of muscle growth potentially involved in sarcopenia and, therefore, support GDF15 as a reliable biomarker.

Also, several studies have shown a key role of Tumor Growth Factor β (TGF β) in muscle and adipose tissue (Rahimi et al., 1998; Lafyatis et al., 1991). TGF β seems to be able to inhibit myogenesis in various muscle diseases (Sartori et al., 2014). However its role in sarcopenia is not completely clear. Bone morphogenetic proteins (BMPs) are well-known members of the TGF superfamily. Currently there are 20 known isoforms with different and sometimes contrasting roles on a multitude of cellular processes, including proliferation, migration, survival and differentiation. BMPs also act as growth factors, especially in bone (Ruschke et al., 2012). Recent studies have shown that in skeletal muscle, by competing with the MYO/Activin/ TGF β pathway, BMPs could play a key role in the increase of muscle mass (Massagué et al., 2005; Sartori et al., 2014), even though their involvement in the complex framework of sarcopenia is not totally clear.

Irisin (IR) is a peptide produced by cleavage of a fibronectin type III domain containing protein 5 (FNDC5) and secreted by the skeletal muscle especially after physical activity, thus supporting the beneficial effects of exercise (Boström et al., 2012). Other evidences suggest that it is also secreted by adipose tissue, hence IR could be defined an adipomyokine (Roca-Rivada et al., 2013). Interestingly, a direct relationship between irisin and FST was observed both in obese patients and in healthy people (the same phenomenon was not observed with MYO and activin A). Furthermore it was observed that, in both classes of subjects, irisin mRNA expression also correlated with FST mRNA expression in muscular biopsies (Vamvini et al., 2013). These data are extremely interesting, as they emphasize the possible existence of a subtle relationship mutually adjusted, between FST and irisin in skeletal muscle. Interestingly, a negative relationship between irisin and sclerostin, a protein implicated in the complex mechanisms of bone remodeling,

has also been described in adults with prediabetes (Klangjareonchai et al., 2014). Sclerostin is produced by osteocytes and seems to have an inhibitory action on Wnt-related signaling pathway, thereby reducing osteoblasts differentiation and bone deposition and, also, adipogenesis (Sapir-Koren and Livshits, 2014). Hence, this close connection observed between irisin and sclerostin suggests a captivating theory about a correlation between muscle, fat and bone metabolism in which irisin would play a key role (Raschke et al., 2013; Hofmann et al., 2014). In spite of the need for further studies, these preliminary data on irisin indicate it as a potential marker of sarcopenia.

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin able to induce a production of growth factors associated with differentiation, plasticity and, of course neuronal growth. As it has been detected in primary human myotubes, it can be considered to all intents and purposes a myokine (Raschke et al., 2013). In skeletal muscle, BDNF has been found to be involved in the regulation and survival of motoneurons, but it also seems to have an additional role in the development and differentiation of myoblasts and in the modulation of myocardial function (Raschke and Eckel, 2013; Sakuma et al., 2015; Feng et al., 2015). Other studies highlighted the role of BDNF in the interaction between immune cells and muscle cells (Brunelli et al., 2012; Papathanassoglou et al., 2015). Interestingly, BDNF is also secreted by muscle cells in response to muscle contraction, pointing to its ability to affect all the most important mechanisms related to the functional maintenance of skeletal muscle fibers, such as the processes of differentiation, repair and regeneration (Raschke et al., 2013). In addition to this intriguing paracrine mechanism, BDNF also seems to act via endocrine action, participating in the process of oxidation of fatty acids, and, as already described, in the immune and inflammatory control (Pedersen, 2013).

2.4. Muscle protein turnover

An early sign of sarcopenic damage would be detected by early structural alterations of the muscle (see Table 1). “Neopeptides” are peptides produced from a pre-existing molecule through a series of post-transduction modifications that include processes of glycosylation, phosphorylation, acetylation, methylation and many others, and are formed through a process of cleavage or addition of different chemical groups depending on the affected tissue. The most important neopeptides in the evaluation of muscle mass are serum sarcomeric proteins such as actin, myosin, troponin and tropomyosin, and extracellular matrix proteins (Nedergaard et al., 2013a). Other potential markers for the loss of muscle could be the peptides deriving from the turnover of collagen type VI, such as a type VI collagen N-terminal globular domain epitope (IC6) and an MMP-generated degradation fragment of collagen 6, (C6M). Collagen type VI is present in the basement membrane of many cells, but especially in the sarcolemma. Genetic defects for this type of collagen are generally linked to very serious muscular diseases, such as muscular dystrophy, highlighting the importance of this protein within the maintenance of muscle trophism. Hence, it has been proposed as a biomarker of muscular tissue damage (Nedergaard et al., 2013b).

In the phase of muscle remodeling, another type of collagen, namely type III collagen plays a main role in providing the structural basis for the correct positioning and development of myoblasts. Collagen type III is synthesized from the cleavage of the N and C-terminal portions of its precursor, procollagen type III. During collagen type III synthesis, the N-terminal peptide (P3NP) is released in the serum. P3NP being a by-product of collagen synthesis, it reflects with good confidence the current manners of muscle remodeling, unlike other indicators such as testosterone, GH and IGF 1 which represent purely a hormonal arrangement, and not necessarily reflect the implementation of an anabolic process (Bhasin et al., 2009). Thus, P3NP is measurable in serum, and seems to be a useful marker for the anabolic response to hormones such as testosterone and growth hormone, and it seems associated to variations of the appendicular muscle lean mass (Bhasin et al., 2009).

3-Methylhistidine (3MH) is another molecule that may be implicated in the pathophysiology of sarcopenia. It results from the methylation of histidine residues of actin and myosin, and is able to induce proteolysis of myofibrils (Young and Munro, 1978). 3MH can be measured in urine or plasma, although it is necessary to stop patients from eating meat during the 3 days prior to urine or blood samples collection, as meat intake could invalidate the results (Young and Munro, 1978). Its potential use as a biomarker is supported by an interesting study, in which 3MH, labeled with a nonradioactive isotope was administered orally to healthy subjects, and the following day urine and plasma samples were collected and analyzed by mass spectrometry, in order to get information about myofibrillar proteolysis (Sheffield-Moore et al., 2013).

Skeletal muscle-specific isoform of troponin T (sTnT) may be used as a marker of muscle wasting. Normally small traces of these proteins are present in the circulation as an expression of normal muscle turnover or minor damage (Chase et al., 2013). The presence of significant troponin levels in the blood are an expression of muscle damage. Skeletal muscles are surrounded by multiple layers of connective tissue, thus the disruption of these membranes may explain the presence of troponin, (especially cTnT) in serum, which is to be interpreted as pathological. Interestingly after a 10-week strength-training regimen in community-dwelling elderly subjects, a significant improvement in physical performance was recorded accompanied by a 2-fold decrease of serum levels of cTnT (Abreu et al., 2014), opening new scenarios about this biomarker in sarcopenia and in other pathophysiological conditions.

Finally, serum creatinine level is a parameter to be considered constantly, as it is an indicator of the state of skeletal muscle. Its high accessibility, diffusion and low cost make it play a key role in the global assessment of the elderly sarcopenic patient (Patel et al., 2013). The evaluation of creatinine as a parameter of sarcopenia may be further expanded through the use of the liquid chromatography–tandem mass spectrometry based on D3-creatine dilution method. This exam allows, after providing an oral dose of D₃-creatine, detection of the urinary creatinine increase by isotope ratio mass spectrometry (Stimpson et al., 2013). Urinary creatinine measurements provide an estimate of its precursor, creatine, which, in the human body, originates almost exclusively from striated muscle. As creatinine excretion fluctuates during the day, it is very important to carry out a prolonged urine collection; furthermore, for a more realistic quantification, patients' diets should be meat-free (Proctor et al., 1999).

2.5. Behavior-mediated pathways

Behavioral factors, such as the degree of physical activity, nutritional status and obesity are very important in the onset of sarcopenia. Sarcopenia is well known to be linked to a low degree of physical activity, and it has been shown that exercise, especially resistance training, increases muscle mass and strength in older adults. For this reason physical training programs have been proposed as countermeasure for sarcopenia and dynapenia (Montero-Fernández and Serra-Rexach, 2013) (See Table 1). Interestingly, several blood markers are affected by the degree of physical activity.

Aging-induced elevation in Complement protein C1q secretion, by activation of Wnt signaling pathway in muscles, leads to the development of muscle fibrosis. It was demonstrated that serum C1q level reflects the loss of muscle mass and strength in aging and responds to the effect of progressive physical training, playing a role in the beneficial effect of resistance exercise training in sarcopenia (Watanabe et al., 2015).

Nutritional factors are very important in the development of sarcopenia, and undernutrition biomarkers may help to recognize this condition. At this regard, the presence of anemia with low level of hemoglobin or low serum levels of albumin or selenium may have an influence on physical performance in older persons mainly related to

lower muscle mass and strength (Penninx et al., 2004a; Schalk et al., 2005; Visser et al., 2005; Lauretani et al., 2007).

Leptin plays a range of pathophysiological roles in a multitude of body organs and systems. It is produced by adipocytes and it appears to have an effect on skeletal muscle, in particular modulating lipolysis and insulin sensitivity. Leptin receptors are down-regulated by leptin itself, but even insulin resistance is involved in their regulation (Sell et al., 2006). If we consider that the muscle is the major consumer of glucose, the presence of sarcopenia may be a risk factor for the development of insulin resistance (Zamboni et al., 2008). In sarcopenia a reduction of leptin receptors along with a decrease in muscle mass has been observed, with consequent increase of circulating levels of leptin. This interesting parallel between leptin and sarcopenia supports the hypothesis that leptin may be involved primarily in the development of sarcopenic obesity, more than simple obesity. Exogenous leptin has proven capable of reducing protein synthesis in myocytes, suggesting that it has a main role in the development of sarcopenia (Argilés et al., 2005; Fuentes et al., 2010; Hamrick et al., 2010). Moreover, it is important to highlight that leptin has the ability to cause inflammation (Martin et al., 2008), as well as to negatively regulate the levels of IGF 1 and testosterone (Proctor et al., 1998), well-known factors involved in the development of sarcopenia.

Higher plasma levels of uric acid were observed in older men and women with higher handgrip (Macchi et al., 2008) and, similarly, a positive relationship between higher magnesium serum levels and indexes of muscle performance like lower-leg muscle power and grip strength was identified (Dominguez et al., 2006). Moreover, low values of serum 25-hydroxy vitamin D level are associated not only with a low physical performance in the elderly, but also with muscle metabolism and mass decrease, resulting in lower grip strength (Visser et al., 2003; Houston et al., 2007).

2.6. Inflammation-mediated pathways and redox-related factors

It is well known that the adipose tissue, whose relative percentage often increases in association with sarcopenia, secretes a huge number of pro-inflammatory cytokines, such as interleukins (IL-6, IL-1) and tumor necrosis factor alpha (TNF-alpha), all found to be related to aging processes and, accordingly, to sarcopenia (Visser et al., 2002). These cytokines play a key role in determining sarcopenia for a direct harmful effect on skeletal muscle by developing lower physical performance and muscle strength in the elderly, and consequently disability (Ferrucci et al., 1999; Cesari et al., 2004; Penninx et al., 2004b) (see Table 1).

Interleukin 6 (IL-6), a well known proinflammatory cytokine, was one of the first “myokines” to be identified. It is secreted by both type 1 and 2 muscle fibers *in vitro*, while IL-6 plasma concentrations after exercise are higher than in resting conditions (Pedersen and Hojman, 2012). High levels of IL-6 can be paradoxically associated to a reduction of the anti-inflammatory effects of cytokines such as IL-10 (Michaud et al., 2013). This emphasizes the complexity of the so-called “inflammaging” process. It was also demonstrated that patients suffering from obesity and diabetes who develop sarcopenic obesity in older age, show persistently and markedly elevated levels of proinflammatory cytokines including IL-6 (Sell et al., 2012), demonstrating a subtle correlation between endocrine and metabolic phenomena and inflammaging.

Also, it has been shown that inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF α) and Interleukin 1 (IL-1) are capable of blocking the differentiation of myoblasts only in the presence of an up-regulation of activin (Trendelenburg et al., 2012). The synergic activin-inflammatory cytokines axis was confirmed in models of age-related sarcopenia. The hypothesis of the existence of a cytokine/activin pathways axis suggests a new interesting scenario about mechanisms by which inflammatory cytokines influence skeletal muscle, and offers a

convincing explanation of the physiological role of this pathway in the impaired muscle homeostasis observed in sarcopenia.

Another interesting potential biomarker of sarcopenia could be Butyryl-cholinesterase (α -glycoprotein synthesized in the liver, b-CHE). We have recently demonstrated that b-CHE, a routinely marker of chronic inflammation and malnutrition, is linearly related with grip strength and muscular mass in elderly subjects (Cacciatore et al., 2015).

Oxidized low-density lipoprotein (oxLDL), markers of lipoprotein peroxidation and protein carbonyls, and therefore, markers of oxidative damage, are associated with mobility limitation and grip strength decrease in older persons (Cesari et al., 2005; Howard et al., 2007). In contrast, antioxidant substances, like carotenoids and vitamin C, and circulating levels of alpha- and gamma-tocopherol seem to be inversely correlated with sarcopenia determinants (Semba et al., 2007). Intriguingly, sarcopenia increases the infiltration of immune cells into injured muscles, and therefore, activated immune cells and injured muscles release proinflammatory mediators and reactive oxygen and nitrogen species (RONS) via lipoxygenase, NADPH oxidase, xanthine oxidase, and inducible nitric oxide synthase leading to oxidative stress (Sallam and Laher, 2016).

3. Multifactorial model

Ideal biomarkers of sarcopenia should be valid, reproducible, reliable, specific, inexpensive and easily accessible. Until now, a valid and unique biomarker of sarcopenia has not yet been identified. Indeed, the “multifactorial” pathogenesis and the multitude of pathways that lead to this condition do not allow for the identification of a single biomarker. Several studies have proposed a number of molecules potentially involved in the pathogenesis of sarcopenia that may reveal very promising in the future. Interestingly, an inverse association among six circulating biomarkers and physical performance explored with gait speed has been recently observed. In particular, IL-6 (weighted $r = -.22$) and tumor necrosis factor receptor 2 (weighted $r = -.19$) showed the greatest significant correlations (Peterson et al., 2016). Yet, a multivariate approach was applied to explore the relationship between a panel of inflammatory biomarkers and gait speed in a sample of older community dwellers including 14 inflammatory markers, growth factors, and vascular adhesion molecules, related to systemic and/or vascular inflammation measured via a multiplex, magnetic bead-based immunoassay (Marzetti et al., 2014b; Calvani et al., 2015). Also, partial least squares-discriminant analysis was performed to identify the patterns of inflammatory mediators associated with gait speed categories. A higher level of IL-8 and TNF- α characterized the inflammatory profile of older persons walking slower than 0.8 m/s. These attempts to identify the most powerful sarcopenia-related biomarker have two main limitations: first, the identified biomarkers basically invest only the inflammatory origin of sarcopenia and, second, patients were selected for a deficit of physical performance that does not precisely identify sarcopenia but only one aspect of this syndrome. A speculative model to correctly identify the pathogenesis of sarcopenia may be built by using a panel of biomarkers for different pathological pathways (see Fig. 1). This approach would allow for the identification of single or multiple pathophysiological mechanisms that represent the only way to for a correct management of elderly subject with sarcopenia.

4. Conclusions

Sarcopenia is a geriatric multifactorial syndrome associated with worse clinical outcomes. The identification of the pathogenesis of sarcopenia represents the main goal of a modern approach to understand this intriguing syndrome. Because of the multifactorial genesis of sarcopenia, it is imperative to emphasize the importance of different biomarkers determination for each pathophysiological pathway.

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