Myostatin as a potential biomarker to monitor sarcopenia in hip fracture patients undergoing a multidisciplinary rehabilitation and nutritional treatment: a preliminary study

Alessandro de Sire, Alessio Baricich, Filippo Renò, Carlo Cisari, Nicola Fusco & Marco Invernizzi

Aging Clinical and Experimental Research

e-ISSN 1720-8319

Aging Clin Exp Res
DOI 10.1007/s40520-019-01436-8

Springer

Your article is protected by copyright and all rights are held exclusively by Springer Nature Switzerland AG. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Author's personal copy

Aging Clinical and Experimental Research
https://doi.org/10.1007/s40520-019-01436-8

SHORT COMMUNICATION

Myostatin as a potential biomarker to monitor sarcopenia in hip fracture patients undergoing a multidisciplinary rehabilitation and nutritional treatment: a preliminary study

Alessandro de Sire[1,2] · Alessio Baricich[1,3] · Filippo Renò[4] · Carlo Cisari[1,3] · Nicola Fusco[5] · Marco Invernizzi[1]

Received: 6 November 2019 / Accepted: 28 November 2019
© Springer Nature Switzerland AG 2019

Abstract
Hip fractures are the most common osteoporotic fractures related to disability in older adults, requiring surgery and a subsequent rehabilitation treatment. Sarcopenia is currently considered as a predictive of worse outcome in hip fracture patients and myostatin has been recently proposed a potential biomarker of this condition. Twenty hip fracture patients after total hip replacement (mean aged 75.9 ± 2.4 years) were randomly divided into two groups of ten subjects (groups A and B). Both groups performed a rehabilitation program (5 sessions of 40 min/week for 2 weeks, followed by home-based exercise protocol). Group A received also 2-month amino acid supplementation. Serum myostatin levels significantly decreased after 2 months in both group A ($p = 0.01$) and group B ($p = 0.03$) in sarcopenic patients only in group A ($p = 0.04$). These results suggest that myostatin might be considered a promising biomarker of sarcopenia in hip fracture older adults' patients undergoing rehabilitation and amino acid supplementation.

Keywords Myostatin · Sarcopenia · Hip fractures · Rehabilitation · Nutrition therapy · Amino acids

Background

During the past few years, there has been an increasing interest in the definition of clinically relevant biomarkers of aging and sarcopenia [1]. In this scenario, osteoporotic hip fractures are the most common fragility fractures in the elderly and are associated with increased mortality (33% at 1 year) and disability in older people, requiring surgery and subsequent rehabilitative treatment [2].

Sarcopenia is a degenerative disorder and is characterized by the progressive loss of muscle mass with an associated deficit of strength and decreased physical performance; given the crucial role of skeletal muscles in bone function and repair, sarcopenia has been widely proposed as a risk factor for hip fractures, being also considered predictive of worse outcomes in these patients [3]. The pathogenesis of sarcopenia is linked to an alteration of the homeostasis between protein anabolism and catabolism in the muscle tissue, resulting in the progressive reduction of the muscle mass. This homeostasis is fostered by several factors, such as inflammatory cytokines, cortisol, myostatin, and the over-expression of the ubiquitin–proteasome pathway, which promotes muscle tissue degradation, while adequate protein/amino acid intake, growth hormone (GH), and insulin growth factor (IGF)-1, instead, promote the synthesis of new muscle tissue. Importantly, the strong relationship between osteoporosis and sarcopenia was pointed out, underlining how these two conditions share several pathogenic factors, as confirmed by the documented action of muscle-derived cytokines (myokines) that might influence bone density, growth, and repair [4]. Despite several circulating factors have been proposed to predict and monitor skeletal muscle

✉ Marco Invernizzi
marco.invernizzi@med.uniupo.it

1 Physical and Rehabilitative Medicine, Department of Health Sciences, University of Eastern Piedmont "A. Avogadro", Viale Piazza D'Armi, 1, 28100 Novara, Italy

2 Rehabilitation Unit, "Mons L. Novarese" Hospital, Moncrivello, Vercelli, Italy

3 Physical Medicine and Rehabilitation Unit, University Hospital "Maggiore della Carità", Novara, Italy

4 Innovative Research Laboratory for Wound Healing, Department of Health Sciences, University of Eastern Piedmont "A. Avogadro", Novara, Italy

5 Division of Pathology, IEO-European Institute of Oncology IRCCS, University of Milan, Milan, Italy

Published online: 14 December 2019

Author's personal copy

modifications in older adults, there is still a lack of a "gold standard" biomarker to date.

The growth/differentiation factor (GDF)-8, also known as myostatin, is an extracellular cytokine belonging to the transforming growth factor β (TGF-β) superfamily; given its key role in muscle catabolism, it has been proposed as a biomarker of age-related sarcopenia [4]. It should be noted, however, that the relationship among the levels of circulating myostatin mRNA and aging modifications is still not demonstrated and still subject of debate. However, several studies showed that hip fractures might influence serum levels of myostatin, because TGF-beta superfamily seems to be actively involved in fracture healing [5, 6].

It has been shown that physical exercise could impact the fine processes of myocyte homeostasis regulation, leading to a boost of the anabolic processes, independently of the different type of physical exercise protocol performed [7, 8]. Finally, the effect of amino acid supplementation on myostatin serum levels is still largely unknown, considering the few studies in literature and the heterogeneity of the supplementation protocols [9, 10]; in particular, amino acid supplementation was previously used for 4 months in a pilot study showing to be a good therapeutic option in antagonizing malnutrition in patients affected by chronic renal failure undergoing haemodialysis [11]. Up to date, the combined approach of exercise and dietary supplements is still the basis for the clinical management of sarcopenia [12, 13]. In addition, to the best of our knowledge, there are no studies investigating on the relationship between serum myostatin and hip fracture undergoing a multidisciplinary rehabilitation and nutritional treatment.

In this scenario, the aim of the current study was to characterize the role of myostatin as a promising biomarker of sarcopenia and skeletal muscle modifications in older adults with osteoporotic hip fracture undergoing a multidisciplinary rehabilitation and nutritional treatment.

Methods

Data from a sample of osteoporotic hip fracture patients (age > 65 years at 3 months after hip fracture) subjected to total hip replacement were retrieved. Twenty patients were randomly selected from the study population of a recent pilot randomized controlled trial (RCT) performed by our working group, showing the effects of a combination of essential amino acid supplementation and rehabilitation on physical functioning in hip fracture patients after a total hip replacement [14].

As showed by the flow chart in Fig. 1, the 20 participants were randomized into 2 groups of 10 subjects, namely, Group A and Group B. Moreover, as described in the recent pilot RCT [14], the participants were also divided into sarcopenic and non-sarcopenic patients, according to European

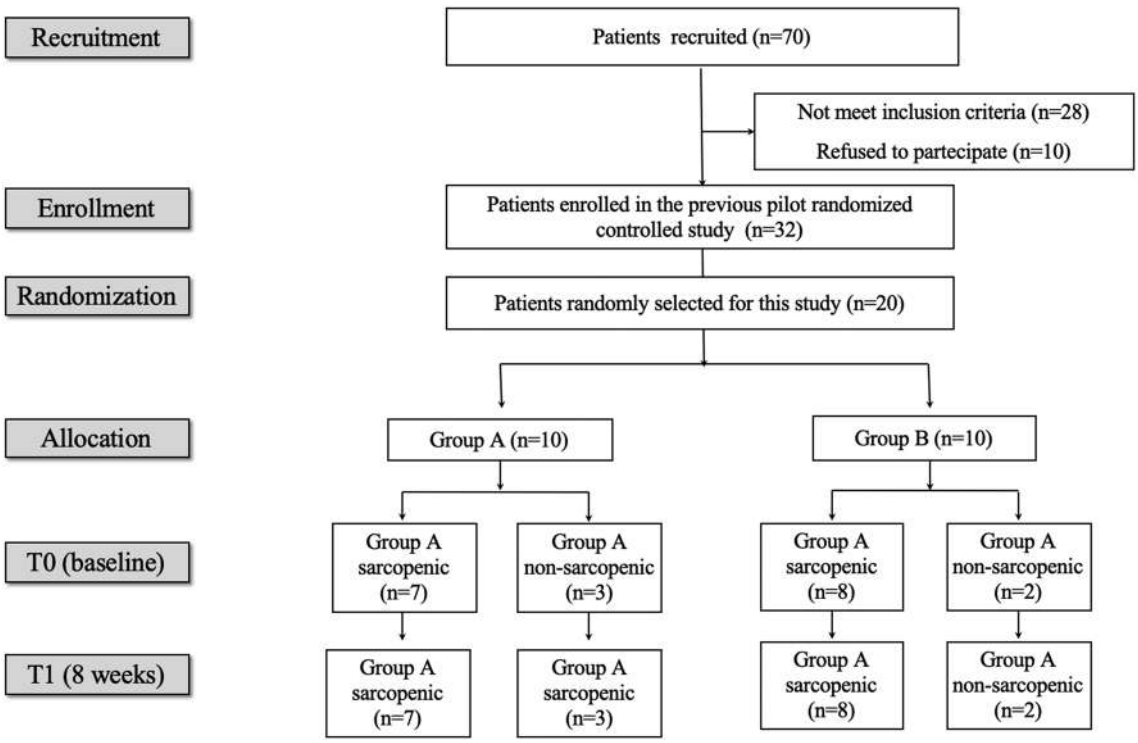

Fig. 1 Study flow chart

Author's personal copy

Working Group on Sarcopenia in Older People (EWGSOP) criteria [15], considering the following parameters [14]: skeletal muscle mass index (SMI), obtained by whole-body tetrapolar bioelectrical impedance analysis (BIA 101 Anniversary Sport Edition—ASE—Akern Srl; Florence, Italy); appendicular muscle strength, measured by hand grip strength test (hand-held Jamar® dynamometer); and physical performance, using the Timed Up and Go test (TUG).

All patients performed a specific physical exercise rehabilitative program, consisting of 5 sessions of 40 min each (15 min of walking training, 10 min of upper and lower limb strengthening and stretching, and 10 min of balance exercises) per week for 2 weeks under the supervision of an experienced physical therapist, and, subsequently, a home-based exercise protocol (aerobic, flexibility, resistance, and neuromotor) without physical therapist supervision [14]. All patients in group A received a 2-month amino acid supplementation (Aminotrofic®, Errekappa Euroterapici, Milan, Italy), two sachets of 4 g daily (1250 mg of L-leucine, 650 mg of L-lysine; 625 mg of L-isoleucine, 625 mg of L-valine, 350 mg of L-threonine, 150 mg of L-cystine, 150 mg of L-histidine, 100 mg of L-phenylalanine, 50 mg of L-methionine, 30 mg of L-tyrosine, 20 mg of L-tryptophan; 0.15 mg of vitamin B6, and 0.15 mg of vitamin B1) [14].

At the baseline (3 months after hip fracture) and at the end of 2-month treatment, the myostatin serum levels were evaluated in all the participants, undergoing two blood samples, centrifuged for 15 min at 3000 rpm, with the resulting serum stored in the freezer at − 20 °C. Circulating serum levels of myostatin were measured using the enzyme-linked immunosorbent assay (ELISA) Kit (Human Myostatin ELISA Kit MyBioSource®, number MBS703668), consisting of 96 wells, 6 standard samples with known myostatin concentrations, an Horseradish Peroxidase conjugate, substrates A and B and a solution for the stop of the reaction. Intra-group differences of serum myostatin levels were evaluated with Wilcoxon matched pairs signed rank test, whereas differences between groups were evaluated with the Mann–Whitney *U* test. A type I error level of 0.05 was chosen.

Results

The 20 participants (mean aged 75.9 ± 2.4 years) were randomized into 2 groups: group A ($n = 10$; mean aged 76.1 ± 2.2 years) and group B ($n = 10$; mean age 75.7 ± 2.6 years), with a similar prevalence of sarcopenia (group A: 70% vs group B: 80%). Baseline characteristics are shown in Table 1. Serum myostatin levels significantly decreased after 2 months of treatment in both group A (1.2 ± 0.2 vs 0.9 ± 0.3 ng/mL; $p = 0.01$) and group B (1.3 ± 0.3 vs 1.1 ± 0.4 ng/mL; $p = 0.03$). Interestingly, a significant reduction of serum myostatin levels was observed in the sarcopenic patients of group A (1.3 ± 0.3 vs 0.9 ± 0.5 ng/mL; $p = 0.04$), but not in those in group B (1.2 ± 0.6 vs 1.0 ± 0.7 ng/mL; $p = 0.12$). There were no statistically significant differences between groups in terms of serum myostatin levels. Data have been graphically resumed in Fig. 2.

Table 1 Baseline characteristics of study population

	Group A ($n = 10$)	Group B ($n = 10$)	*p* value
Age (years)	80.33 ± 6.72	77.65 ± 8.40	NS
Sex (male/female)	1/9	2/8	NS
Myostatin levels (ng/mL)	1.3 ± 0.3	1.2 ± 0.6	NS
Sarcopenic patients	7 (70.0)	8 (80.0)	NS

Continuous variables are expressed as means ± standard deviations; categorical variables are expressed as counts (percentages); rates are expressed as *x*/*y*

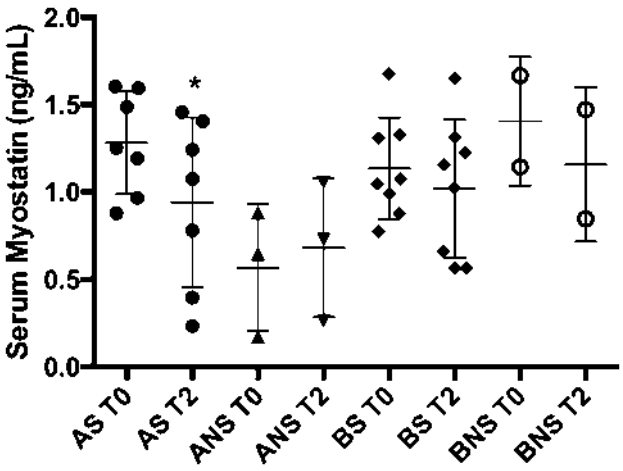

Fig. 2 Serum myostatin modifications in group A and group B patients. *AS* group A sarcopenic, *ANS* group A non-sarcopenic, *BS* group B sarcopenic, *BNS* group B non-sarcopenic. *$p < 0.05$

Discussion

In this proof of principle study, we found a significant intra-groups difference in terms of serum myostatin levels in both groups (A and B), according to the previous data in literature [7, 8]. On the other hand, we found no significant differences between groups in serum myostatin levels at the end of treatment, probably due to the correlation between physical exercise protocol and myostatin levels, independently from amino acids supplementation.

The main limitation of this study was the small sample size, in particular considering the sub-groups (sarcopenic and non-sarcopenic); furthermore, the lack of data on

Author's personal copy

nutritional levels at baseline and a long-term follow-up could be considered as study limits.

In conclusion, even though on a small sample, we showed for the first time in literature that a 2-month combined nutritional and rehabilitative intervention in older adults with surgically treated hip fracture induced a reduction in serum myostatin levels, suggesting its potential role as a promising circulating biomarker to monitor sarcopenia in older adults.

Further prospective clinical studies on larger cohorts of patients, coupled with cutting-edge molecular analysis of circulating myostatin-related biomarkers (e.g., mRNA, miRNAs) are warranted to confirm and broaden the clinical relevance of our discoveries.

Funding The study was not funded.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical statement and Informed consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

References

1. Calvani R, Picca A, Marini F et al (2018) The "BIOmarkers associated with Sarcopenia and PHysical frailty in ElderRly pErsons" (BIOSPHERE) study: rationale, design and methods. Eur J Intern Med 56:19–25
2. Tarantino U, Capone A, Planta M et al (2010) The incidence of hip, forearm, humeral, ankle, and vertebral fragility fractures in Italy: results from a 3-year multicenter study. Arthritis Res Ther 12:R226
3. Cruz-Jentoft AJ, Bahat G, Bauer J et al (2019) Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48:16–31
4. White TA, LeBrasseur NK (2014) Myostatin and sarcopenia: opportunities and challenges—a mini-review. Gerontology 60:289–293
5. Cho TJ, Gerstenfeld LC, Einhorn TA (2002) Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res 17:513–520
6. Guo W, Flanagan J, Jasuja R et al (2008) The effects of myostatin on adipogenic differentiation of human bone marrow-derived mesenchymal stem cells are mediated through cross-communication between Smad3 and Wnt/beta-catenin signaling pathways. J Biol Chem 283:9136–9145
7. de Souza EO, Tricoli V, Aoki MS et al (2014) Effects of concurrent strength and endurance training on genes related to myostatin signaling pathway and muscle fiber responses. J Strength Cond Res 28:3215–3223
8. Binns A, Gray M, Henson AC et al (2017) Changes in lean mass and serum myostatin with habitual protein intake and high-velocity resistance training. J Nutr Health Aging 21:1111–1117
9. Becker C, Becker C, Lord SR et al (2015) Myostatin antibody (LY2495655) in older weak fallers: a proof-of-concept, randomised, phase 2 trial. Lancet Diabetes Endocrinol 3:948–957
10. Paoli A, Pacelli QF, Neri M et al (2015) Protein supplementation increases postexercise plasma myostatin concentration after 8 weeks of resistance training in young physically active subjects. J Med Food 18:137–143
11. Sukkar SG, Gallo F, Borrini C et al (2012) Effects of a new mixture of essential amino acids (Aminotrofic®) in malnourished haemodialysis patients. Med J Nutr Metab 5:259–266
12. Iolascon G, Moretti A, de Sire A et al (2018) Pharmacological therapy of sarcopenia: past, present and future. Clin Cases Miner Bone Metab 15:411–419
13. de Sire A, Invernizzi M, Lippi L et al (2019) Nutritional supplementation in hip fracture sarcopenic patients: a narrative review. Clin Cases Miner Bone Metab. 16:27–30
14. Invernizzi M, de Sire A, D'Andrea F et al (2019) Effects of essential amino acid supplementation and rehabilitation on functioning in hip fracture patients: a pilot randomized controlled trial. Aging Clin Exp Res 31:1517–1524
15. Cruz-Jentoft AJ, Baeyens JP, Bauer JM et al (2010) Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing 39:412–423

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.