REVIEW

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Biomarkers for physical frailty and sarcopenia: state of the science and future developments

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Abstract

Physical frailty and sarcopenia are two common and largely overlapping geriatric conditions upstream of the disabling cascade. The lack of a unique operational definition for physical frailty and sarcopenia and the complex underlying pathophysiology make the development of biomarkers for these conditions extremely challenging. Indeed, the current definitional ambiguities of physical frailty and sarcopenia, together with their heterogeneous clinical manifestations, impact the accuracy, specificity, and sensitivity of individual biomarkers proposed so far. In this review, the current state of the art in the development of biomarkers for physical frailty and sarcopenia is presented. A novel approach for biomarker identification and validation is also introduced that moves from the 'one fits all' paradigm to a multivariate methodology.

Keywords Ageing; Disability; Physical performance; Circulating markers; Imaging; Multivariate modelling

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Physical frailty and sarcopenia: the two sides of the same 'impaired' coin

One of the most notable changes in body composition that accompanies ageing is the loss of skeletal muscle mass, originally termed sarcopenia by Rosenberg in 1989.[1] Sarcopenia and its clinical correlates involve impairments in strength, limitations in function, and ultimately physical disability, institutionalization, and mortality.[2,3] Frailty is the term used to indicate a geriatric syndrome characterized by reduced homeostatic reserves, which exposes the individual at increased risk of negative health-related events.[4,5] In particular, the physical frailty (PF) phenotype operationalized by Fried *et al.*[6] has shown to well serve as a predictor of major negative outcomes. It is noteworthy that the PF phenotype shows substantial overlaps with sarcopenia. Indeed, many of the adverse outcomes of PF are believed to be mediated by the muscle decline.[7] From this perspective, sarcopenia may be considered both the biological substrate for the development of PF and the pathway through which the negative health outcomes of PF ensue.[8,9] PF and sarcopenia are, therefore, intimately interconnected and characterized by a unique core condition, that is, physical function impairment.[7]

Over the last decades, geriatrics and gerontology researchers have devoted an increasing amount of efforts in the attempt of designing, developing, and implementing preventive interventions against these 'twin' conditions. The accomplishment of such task has been hampered by the lack of a unique, standardized, and universally agreed operational definition for both PF and sarcopenia. These definitional ambiguities are also reflected by the absence of reliable biomarkers that could be utilized in clinical and research settings to identify the two conditions, track their progression over time, and monitor their response to interventions.[10] Another critical issue in the field of biomarker development resides in the intrinsic complexity of PF and sarcopenia, as evidenced by the large spectrum of phenotypes they

encompass. This aspect has considerable impact on the accuracy, specificity and sensitivity of the parameters that have so far been proposed as biomarkers for the two conditions.

This review presents the current state of the art in the field of biomarkers for PF and sarcopenia. A novel approach for biomarker identification and validation is also proposed that moves from the 'one fits all' paradigm to a multivariate methodology.

Biomarkers for PF and sarcopenia: seeing the tree for the forest?

A biomarker is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'.[11] Hence, an ideal biomarker should support the diagnosis, facilitate the tracking of the condition of interest over time, and assist healthcare professionals in clinical and therapeutic decision-making.[12]

Taking these considerations into account, candidate biomarkers for PF and sarcopenia may be distinguished in four major classes: (i) *antecedent* biomarkers to estimate the risk of developing these conditions; (ii) *diagnostic* biomarkers to detect clinically manifest PF and sarcopenia; (iii) *staging* biomarkers to describe categories or severity of PF and sarcopenia; and (iv) *prognostic* biomarkers to predict the risk of developing adverse health outcomes related to PF and sarcopenia (e.g. mobility disability).[11]

As suggested by the International Working Group on Sarcopenia,[12] several imaging, functional, and biological parameters are potentially able to track single aspects of PF and sarcopenia. The intrinsic (e.g. accuracy, specificity, and sensitivity) and extrinsic (e.g. cost, availability, and time to be performed) properties of each biomarker depend on its specific characteristics (e.g. the mechanisms/processes/parameters measured) and largely drive its potential implementation in screening, baseline evaluation, and/or definition of outcomes.[12]

With respect to imaging biomarkers, although a 'gold standard' technique for the quantification of muscle mass is currently lacking, magnetic resonance imaging (MRI), computed tomography (CT), and dual energy X-ray absorptiometry (DXA) provide an objective and sufficiently reliable measure of muscle or fat-free mass of which muscle comprises the majority.[12] Unfortunately, such imaging equipments are not immediately available in primary care (e.g. the general practitioner's office), which represents the first diagnostic contact for the majority of sarcopenic elderly. In addition, MRI and CT are rather expensive and technically difficult tests. Each of these techniques also provides different estimates of the body composition profile in terms of explored anatomical regions, applied methods and units, and accuracy in defining thresholds of risk, making difficult (if not impossible) direct comparisons across their results. These and other drawbacks limit their use in routine clinical applications.

Noticeably, the muscle mass represents only one of the multiple dimensions of PF and sarcopenia.[13] Mobility decline (resulting from the improper functioning of muscles, coordination, and balance) and, at a broader level, physical function impairment are clear manifestations of ageing that significantly affect the quality of life.[14] Physical function can easily be measured in an objective way through validated assessment tools.[15] Such instruments include the short physical performance battery,[16] the 4-m usual gait speed,[17] the handgrip strength,[18] and the lower extremity muscle power.[2] These tests, however, can be markedly influenced by comorbidities that are often present in older persons, including degenerative or inflammatory diseases of the musculoskeletal system.[19]

While the combined assessment of muscle mass and function is an essential requirement for the identification of sarcopenia, one of the current biggest uncertainties resides in the definition of the thresholds for distinguishing 'physiologic' from 'pathologic' muscle ageing.[13] This limits the applicability of imaging and functional biomarkers in clinic and research settings. In this scenario, the development of biological markers that can be measured in biofluids and used in a cost-effective manner to guide the diagnosis and facilitate the monitoring of PF and sarcopenia would mark a substantial step forward in the healthcare management of older people.[12,20]

The next sections provide a brief overview of several biological markers that have been proposed for PF and sarcopenia, and a critical appraisal of the strengths and weaknesses of the traditional procedures for biomarker development in this field.

The ins and outs of a complex condition

The syndromic nature of PF and sarcopenia as well as the wide range of pathogenic processes that contribute to their development and progression poses major challenges for the identification of specific biological markers. Indeed, the presently available biomarkers for PF and sarcopenia are typically related to specific pathogenic mechanisms and/or phenotypes. As such, they only describe single aspects of the conditions and are weakly associated with clinically relevant outcomes.

Tissular vs. circulating biomarkers

A vast literature exists reporting the involvement of muscle-specific cellular processes in the pathogenesis of sarcopenia

(reviewed in[21]). Examples include deregulation of myocyte apoptosis,[22] derangements in mitochondrial function and quality control,[23,24] oxidative/nitrosative stress,[25] iron dyshomeostasis,[26] and alterations in protein synthesis and breakdown.[27,28] The investigation of such pathways, although providing valuable information on sarcopenia pathophysiology, shows limited clinical applicability.[29] Indeed, the access to muscle tissue requires an invasive procedure (muscle biopsy), which may be perceived as unacceptable by most older persons. This is especially true when considering that biospecimens have to be collected at least at two time-points to determine the progression of the condition or the effects of an intervention. In addition, the dissection of the cellular pathways listed above relies on sophisticated analyses that are not sufficiently standardized and expensive, besides requiring dedicated laboratory equipment and experienced personnel. Moreover, some measurements, such as mitochondrial bioenergetics, need to be performed on fresh tissue and are highly laborious.

Considerable research efforts have therefore been diverted towards the development and validation of blood-borne biomarkers for PF and sarcopenia.[30] The most popular circulating markers are those related to the inflammatory response (e.g. C-reactive protein,[31,32] interleukin 6,[31–34] and tumour necrosis factor α[31,33,35]), clinical parameters (e.g. hemoglobin[31,36] and serum albumin[37]), hormones (e.g. dehydroepiandrosterone sulfate,[38] testosterone,[39] insulin-like growth factor 1,[40] and vitamin D[41]), products of oxidative damage (e.g. advanced glycation end products,[42] protein carbonyls,[43] and oxidized low-density lipoproteins[44]), or antioxidants (e.g. carotenoids[45,46] and α-tocopherol[45]).

Recently, our group demonstrated that telomeres from peripheral blood mononuclear cells were shorter in sarcopenic older persons relative to non-sarcopenic peers, after adjustment for several potential confounders.[47] The relationship between telomere length and sarcopenia appeared to be mainly driven by muscle mass, which may be indicative of a common pathogenic ground for telomere erosion and age-related muscle atrophy. Notably, telomere length was unrelated to either measures of muscle function or the frailty status.

Several circulating biomarkers have been identified in the last years, which may serve as useful parameters to more directly explore skeletal muscle changes in relation to physiological and pathological states. For instance, plasma concentrations of procollagen type III N-terminal peptide (P3NP) may serve as a marker for muscle remodelling elicited by behavioral[48] or pharmacological interventions.[49,50] P3NP is a fragment released by the cleavage of procollagen type III to generate collagen III (a protein produced in soft connective tissues, skin, and muscle), and its levels have been associated with changes in lean mass during testosterone and GH treatment[49,50] or exercise training.[48] In a recent cross-sectional study, linear regression analyses were used to estimate the association between plasma P3NP levels and muscle mass and strength in 687 men and women from the Framingham Offspring Study.[51] Plasma concentrations of P3NP were found to be inversely related to total and appendicular lean mass in postmenopausal women, but not in old men, therefore potentially restricting the use of P3NP as a gender-specific biomarker for muscle mass.

Several studies suggest a role for the circulating C-terminal agrin fragment (CAF) as a marker for skeletal muscle mass and function.[48,52–55] Agrin is a heparan sulfate proteoglycan synthesized in motor neurons, transported along axons and released into the synaptic basal lamina of the neuromuscular junction. Here, it induces the assembly of the postsynaptic apparatus, including the clustering of acetylcholine receptors and the stabilization of presynaptic structures.[56] Increases in circulating CAF concentrations are related to neuromuscular junction disruption, which in turn is involved in muscle fibre denervation, atrophy, and dysfunction.[57]

Similar to CAF, plasma levels of extracellular heat shock protein 72 (eHsp72) are inversely associated with muscle mass and function.[58] The production of eHsp72 has been linked with inflammation[59] and motor neuron apoptosis/survival pathways.[60] However, the underlying pathophysiology in the context of sarcopenia is presently unclear.

It is widely recognized that the skeletal muscle acts as a secretory organ through the production and release of cytokines and other peptides (collectively known as 'myokines') with autocrine, paracrine, or endocrine effects.[61] The muscle-cell secretome consists of several hundreds of secreted products. Identified myokines include myostatin, leukaemia inhibitory factor, interleukins 6 and 7, brain-derived neurotrophic factor, insulin-like growth factor 1, fibroblast growth factor 2, follistatin-related protein 1, and irisin.[61] Because the release of myokines from the skeletal muscle might be altered during the development of PF and sarcopenia, these biomolecules could serve as biomarkers for muscle (dys)function.[19] This possibility warrants further investigation.

A novel approach, based on the dilution of an oral dose of creatinine-(methyl-d(3)) (D3-creatine) determined by urine D3-creatinine enrichment, is receiving increasing attention for its potential application as a means to quantify muscle mass.[62] The method has been tested in laboratory rodents[63] and humans[62] and provides estimates of total muscle mass that well correlate with MRI measurements. Under ideal conditions, the performance of the D3-creatine method has the potential to be superior to DXA.[62] Unfortunately, the detection of D3-creatine requires isotope ratio mass spectrometry or liquid chromatography/tandem mass spectrometry technologies, therefore limiting its assessment to well-equipped medical and research centres. In addition, the method only provides estimates of total muscle mass with no information on muscle function.

Single biomarkers for complex conditions: the blind men and the elephant?

The quite long list of candidate circulating biomarkers and their weak association with relevant clinical outcomes highlight the concept that there might not be *one* single biological marker that reliably tracks the multitude of different contributors and phenotypes of PF and sarcopenia. It is conceivable that a given phenotype (e.g. muscle atrophy and weakness) might be the resultant of distinct pathogenic processes. Furthermore, the environment might play a role as well, by potentially triggering different pathophysiologic mechanisms at the basis of the studied phenomenon. Comorbidities (e.g. cardiovascular disease, chronic kidney disease, diabetes mellitus, lung disease, and cancer) may also require consideration when analysing biomarker levels and trajectories. It follows that a single biomarker may not be equally valid from person to person. In addition, PF and sarcopenia develop over years and pathogenic processes may not necessarily be the same during their whole course. Hence, individual biomarkers may be relevant only within limited timeframes.

Bearing these considerations in mind, a shift of paradigm is needed, moving from the quest for a single biomarker to the development of multivariate/multidimensional modelling of a panel of complementary biomarkers (likely within multiple classes: imaging, circulating biomolecules, and functional tests). This approach may promote (i) the early detection of otherwise subclinical conditions; (ii) the diagnostic assessment of clinically manifest PF and sarcopenia; (iii) the risk stratification of subjects with a suspected or confirmed diagnosis; (iv) the tracking of the conditions over time; (v) the selection of an appropriate therapeutic intervention; and (vi) the monitoring of the response to treatment.[64]

The aim, methodology, and characteristics of this novel approach are described in the next sections. We are aware that the following dissertation might result challenging for readers who are not expert in biostatistics. Nevertheless, a fairly detailed description of the methodology is necessary to introduce a new strategy for biomarker development.

A strategy for multivariate biomarker discovery

Given the complex phenotypical and pathophysiological frames of PF and sarcopenia, the single and isolated inspection of variables can result in a partial or incorrect picture. On the other hand, mainly through the implementation of 'omics' disciplines, multivariate analyses have been gaining a more and more relevant role in clinical practice[65] and may easily be extended to the search for PF and sarcopenia biomarkers.

When more than a single index (variable, biomarker) is recorded for each subject, the clinical data can be arranged in a $m*n$ matrix **X**, where m is the number of individuals and n the number of monitored variables (e.g. biomarker(s), age, and gender). Starting from this data matrix, multivariate biomarker discovery relies on the formulation of models, which, depending on the knowledge of subjects and study design, can be exploratory or predictive. The exploratory approach is the only one feasible when (i) the dimension of the studied cohorts is too small to formulate and validate a reliable predictive model or (ii) the interest is focused on the phenomenological characterization of the disease and/or the identification of signatures in the measured variables. To this purpose, many of the tools for multivariate exploratory analysis are based on the concept of 'bilinear modelling'. This implies the possibility that the experimental data matrix **X** can be decomposed into the product of two matrices **T** and **P**, which in turn are chosen to facilitate interpretation and hypothesis generation:

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathrm{T}} \tag{1}$$

The meaning of equation (1) is that one can search for a better representation of the data by projecting them onto a low-dimensional space, the directions of which are selected to meet specific criteria. This allows visualizing similarities and dissimilarities among the individuals by means of two- or three-dimensional scatterplots (the so-called scores matrix **T** collects the values of these new variables calculated for each person). At the same time, the interpretation in terms of the measured indices remains possible through the inspection of the loadings matrix **P**, which is made of the coefficients of the linear transformation relating the original representation to the new one. In particular, when the new directions are chosen so to retain as much as possible of the information contained in the original data matrix **X** (i.e. in mathematical language, to provide the best low-dimensional representation in a least squares sense), the corresponding method is called principal component analysis (PCA).[66] PCA is a widely applicable method also suitable for dealing with the interpretation of clinical data.

However, when multiple sources of variability are present (for instance, because of whether the person is ill or healthy, whether the study is longitudinal, and therefore includes some form of time course or not, or to the presence of age or gender groups), their individual effect can be confounded in the overall PCA model. To overcome this interpretational problem, in the last years, a strategy based on coupling the concepts of analysis of variance with the exploratory power of PCA has been proposed. The method, called ANOVA-simultaneous component analysis (ASCA), is based on the decomposition of the experimental data matrix into the individual contribution related to the main factors controlled in the study design and their interactions, and to further

interpret each of the resulting matrices using a PCA model.[67] For instance, if the data were collected in a population including healthy and sarcopenic frail persons of both genders at different times, the ASCA analysis would proceed by decomposing the resulting data matrix **X** into the contributions:

$$\begin{aligned}\mathbf{X} = {} & \mathbf{X}_{\text{disease}} + \mathbf{X}_{\text{gender}} + \mathbf{X}_{\text{time}} + \mathbf{X}_{\text{disease}\times\text{gender}} \\ & + \mathbf{X}_{\text{disease}\times\text{time}} + \mathbf{X}_{\text{time}\times\text{gender}} \\ & + \mathbf{X}_{\text{disease}\times\text{gender}\times\text{time}} + \mathbf{X}_{\text{res}}\end{aligned} \quad (2)$$

In equation (2), the various terms indicate the matrices accounting for the effect of the different factors and interactions on the experimental indices measured, and $\mathbf{X}_{\text{res}}$ the matrix associated with the variance not explained by the study design. Each of these matrices can then be analysed by PCA, in order to find out how a particular factor (or interaction) may affect the clinical parameters.

When the dimension of the cohort and the study design allow building a reliable predictive model, biomarker discovery can be accomplished through the construction and validation of an appropriate classification model.[68] The aim of classification methods is to build a model that, based on the values of the measured variables, allows assigning an individual to one or more categories, the category being described as a group of (in this case) people, sharing similar characteristics. In particular, for the sake of biomarker discovery, one would probably consider a setup comprising two categories: healthy and sarcopenic frail elderly. Accordingly, building a classifier would mean using the available data to formulate a predictive model that should be able to forecast, based on a set of measurements collected on the subject, whether he/she presents or not the condition of interest. In order for the model to be of any relevance, a key role is played by the validation step. In the validation phase, the model is applied to a set of individuals whose real category is known but that are treated in a blind fashion. By comparing the real to the predicted outcome, it is indeed possible to estimate the reliability and accuracy of the model. If the model is validated, the inspection of the model parameters allows the formulation of hypotheses about possible biomarker candidates and their mutual correlation.[69]

Here, it can be stressed that, also in the context of predictive model building, the possibility of using methods based on the bilinear concept described previously (e.g. partial least squares-discriminant analysis, PLS-DA) allows coupling the reliability and accuracy of the prediction with the possibility of a low-dimensional representation of the data, which in turn permits an easier and more straightforward interpretation.

Recently, we provided a preliminary example of the analytical approach proposed in the present manuscript. Specifically, a multivariate strategy was applied to explore the relationship between a panel of inflammatory biomarkers and gait speed in a sample of older community dwellers.[70] A panel of 14 inflammatory markers, growth factors, and vascular adhesion molecules, related to systemic and/or vascular inflammation, was measured via a multiplex, magnetic bead-based immunoassay. PLS-DA was subsequently used to identify the patterns of inflammatory mediators associated with gait speed categories. This approach allowed identifying specific profiles of circulating inflammatory markers characterizing older persons with different levels of physical performance. Specifically, participants with gait speed above the critical threshold of $0.8\,\text{m}\cdot\text{s}^{-1}$ were characterized by higher circulating levels of P-selectin, interferon γ, and granulocyte macrophage colony-stimulating factor. Conversely, higher levels of interleukin 8, myeloperoxidase, and tumour necrosis factor α defined the inflammatory profile of older persons walking slower than $0.8\,\text{m}\cdot\text{s}^{-1}$. A robust double cross-validation procedure confirmed the reliability of the PLS-DA model and of the obtained results.

'Emotion recollected in tranquility'

In the previous sections, we briefly described the state of the art and some of the unaddressed issues in the quest for biomarkers for PF and sarcopenia. But what would be the *ideal* strategy to identify biomarkers that could be utilized in the clinical realm? As stated by Cesari *et al.*[12] in the recommendations from the International Working Group on Sarcopenia for the use of biomarkers in clinical trials, 'it is currently difficult to provide long-lasting statements, recommendations, and guidelines', because the study of sarcopenia and frailty still represents a 'work in progress', always amenable to changes and redirections.

The first unavoidable step is the adoption of a unique, objective, standardized, and clinically relevant definition of PF and sarcopenia that is able to capture the multifaceted nature of these geriatric syndromes and facilitate their translation in the clinical arena. As recently suggested by our group,[7] the physical function impairment that occurs in the absence of disability may represent the shared core of PF and sarcopenia. Such a functional deterioration, involving deficits in gait speed, balance, and muscle strength, can be objectively assessed through the short physical performance battery.[16] This conceptualization may optimally serve for (i) a univocal/unambiguous assessment of PF and sarcopenia to be adopted also by public health authorities and regulatory agencies; (ii) the implementation of standardized screening and diagnostic procedures; (iii) the definition of novel targets for interventions against disability; and obviously, (iv) the development of reliable biomarkers. This operationalization of PF and sarcopenia could then allow the selection of a specific 'target' population to tailor treatments and interventions and to assess the validity of

candidate biomarkers. In the multivariate setting proposed in the previous sections, a definite clinical entity could provide clear and measurable outcomes against which to test the sensitivity, specificity, and accuracy of biomarkers, which should be evaluated on an adequate timeframe.

Another point to be addressed concerns the early identification of PF and sarcopenia and the development of primary prevention strategies. When PF and sarcopenia will be definitely framed and reliable biomarkers developed, would it be possible to identify subjects who are at risk to become sarcopenic/physically frail and in whom interventions could be started earlier in life? In this regard, it has been shown that lifestyle habits and physical health during adulthood could determine the rate of muscle strength decline and the development of functional limitations in advanced age.[18,71] For instance, Stenholm *et al.*[71] found that midlife physically strenuous work, excess body weight, smoking, cardiovascular disease, hypertension, diabetes mellitus, and asthma predicted muscle strength decline over 22 years of follow-up. In addition, significant weight loss, becoming physically sedentary, persistent smoking, incident coronary heart disease, diabetes mellitus, chronic bronchitis, chronic low-back pain, long-lasting cardiovascular disease, hypertension, and asthma have been associated in the same study with accelerated decline in handgrip strength.[71] Interestingly, birth weight and pre-pubertal and pubertal growth may affect muscle mass and strength as well as physical performance in late life.[72–74]

Taken together, these findings suggest that individuals at risk of PF and sarcopenia could be identified well before the decline in physical function reaches a critical threshold (*Figure* 1). In this scenario, the multivariate strategy proposed could be used to model imaging, functional, biological, pathological, and pharmacological parameters. At the same time, it might support the computation of a 'sarcopenia and frailty risk score' or a 'sarcopenia and frailty risk chart', similar to what is routinely carried out for other medical conditions (e.g. cardiovascular disease and osteoporosis).[75] Such an approach could allow defining and monitoring the health trajectory and the timely implementation of primary preventive strategies, including nutritional interventions and physical exercise.

Borrowing the language and toolboxes of multivariate statistical process control,[76,77] it could be possible to build a multivariate model of the homeostatic conditions of a person [his/her 'normal operating conditions (NOC)'], which would provide a picture of how all the monitored parameters covary when nothing anomalous is occurring. Then, a longitudinal analysis of the individual time trajectories of the subject could be carried out by inspecting multivariate control charts. An example of such an approach is depicted in *Figure* 2, which shows the trend of squared prediction error (the sum of squares of the difference between the actual values of X and those predicted by the model) as a function of time. The data recorded under NOC are used to define the confidence limits of the statistics (dashed lines in *Figure* 2). These pre-set control limits would allow detecting *at an early stage* the possible onset of a critical condition (e.g. mobility

Figure 1 Possible trajectories of physiological reserve during ageing. In the case of accelerated ageing, the decline in physiological reserve is steeper relative to the successful aging scenario. In this latter case, the development of physical frailty and sarcopenia may be compressed towards the end of life. Critical events (e.g. intercurrent illnesses, hospitalizations, and falls) may cause sudden decreases in physiological reserve, which correspond to proportional changes in biomarker levels. The dashed lines identify the diagnostic cutoffs of biomarkers. The yellow and the red areas correspond to clinically manifest physical frailty/sarcopenia and disability, respectively.

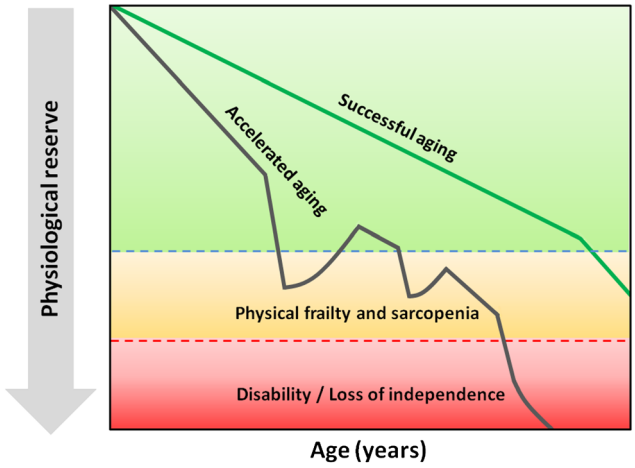
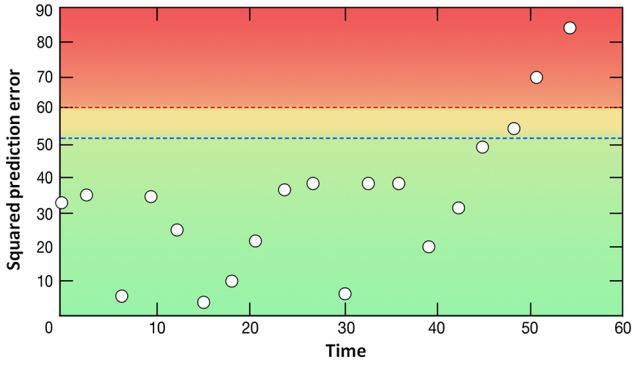


Figure 2 Example of a multivariate control chart (based on PCA or PLS squared X-residuals). The circles depict the resultant of multidimensional assessments over time. The dashed lines correspond to the 95% and 99% confidence limits of the corresponding statistics. Circles above the control limits indicate that the subject is departing from his/her 'normal operating conditions' and may account for the onset of an adverse health-related event (e.g. mobility disability). The building and inspection of a multivariate control chart could allow detecting the onset of a critical condition at a very early stage and the planning of timely interventions.



disability). One main advantage of such multivariate strategy is that it can detect not only anomalous values of specific parameters, but also, and more importantly, even subtle changes in the overall correlation structure among all the measured indices.

Conclusion

Current available biomarkers for PF and sarcopenia are only able to capture single aspects of the conditions and are weakly associated with clinically meaningful outcomes. The adoption of multidimensional/multivariate approaches could help cope with the complex phenotypical and pathophysiological nature of PF and sarcopenia and allow (i) capturing the different domains of the syndromes, (ii) obtaining information about the underlying pathophysiology, and (iii) identifying novel biological targets for preventive or therapeutic interventions.

In a famous passage of the poem 'Little Gidding', T. S. Eliot wrote that 'We shall not cease from exploration and the end of all our exploring will be to arrive where we began and to know the place for the first time'.[78] The quest for biomarkers resembles this 'exploration', at the end of which we may possess the tools necessary to finally decipher the complexity of PF and sarcopenia.

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Conflict of interest

The authors of the present work, with the exception of Federico Marini, are partners of the SPRINTT consortium, which is partly funded by the European Federation of Pharmaceutical Industries and Associations (EFPIA).

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References

1. Rosemberg IH. Summary comments: epidemiological and methodological problems in determining nutritional status of older persons. *Am J Clin Nutr* 1989;**50**:1231–1233.
2. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med* 1995;**332**:556–561.
3. Cruz-Jentoft AJ, Landi F, Schneider SM, Zuniga C, Arai H, Boirie Y *et al*. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age Ageing* 2014;**43**:748–759.
4. Rodriguez-Manas L, Feart C, Mann G, Vina J, Chatterji S, Chodzko-Zajko W *et al*. Searching for an operational definition of frailty: a Delphi method based consensus statement: the frailty operative definition—consensus conference project. *J Gerontol A Biol Sci Med Sci* 2013;**68**:62–67.
5. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *Lancet* 2013;**381**:752–762.
6. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J *et al*. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001;**56**: M146–M156.
7. Cesari M, Landi F, Vellas B, Bernabei R, Marzetti E. Sarcopenia and physical frailty: two sides of the same coin. *Front Aging Neurosci* 2014;**6**:192.
8. Marzetti E, Calvani R, Bernabei R, Leeuwenburgh C. Apoptosis in skeletal myocytes: a potential target for interventions against sarcopenia and physical frailty – a mini-review. *Gerontology* 2012;**58**: 99–106.
9. Landi F, Calvani R, Cesari M, Tosato M, Martone AM, Bernabei R *et al*. Sarcopenia as the biological substrate of physical frailty. *Clin Geriatr Med* 2015.
10. Marzetti E, Buford TW, Bernabei R. Is misuse of the sarcopenia due to a lack of biomarkers? *J Appl Physiol* 2012;**113**:680.
11. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**:89–95.
12. Cesari M, Fielding RA, Pahor M, Goodpaster B, Hellerstein M, van Kan GA *et al*. Biomarkers of sarcopenia in clinical trials—recommendations from the International Working Group on Sarcopenia. *J Cachexia Sarcopenia Muscle* 2012;**3**: 181–190.
13. Marzetti E. Imaging, functional and biological markers for sarcopenia: the pursuit of the golden ratio. *J Frailty Aging* 2012;**1**: 97–98.
14. Cummings SR, Studenski S, Ferrucci L. A diagnosis of dismobility—giving mobility clinical visibility: a Mobility Working Group recommendation. *JAMA* 2014;**311**: 2061–2062.
15. Studenski S, Perera S, Wallace D, Chandler JM, Duncan PW, Rooney E *et al*. Physical performance measures in the clinical setting. *J Am Geriatr Soc* 2003;**51**:314–322.
16. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG *et al*. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;**49**:M85–M94.
17. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M *et al*. Gait speed and survival in older adults. *JAMA* 2011;**305**:50–58.
18. Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD *et al*. Midlife hand grip strength as a predictor of old age disability. *JAMA* 1999;**281**:558–560.
19. Scharf G, Heineke J. Finding good biomarkers for sarcopenia. *J Cachexia Sarcopenia Muscle* 2012;**3**:145–148.
20. Palus S, von HS SJ. Muscle wasting: an overview of recent developments in basic

research. *J Cachexia Sarcopenia Muscle* 2014;**5**:193–198.
21. Marzetti E, Lees HA, Wohlgemuth SE, Leeuwenburgh C. Sarcopenia of aging: underlying cellular mechanisms and protection by calorie restriction. *Biofactors* 2009;**35**:28–35.
22. Marzetti E, Lees HA, Manini TM, Buford TW, Aranda JM Jr, Calvani R *et al*. Skeletal muscle apoptotic signaling predicts thigh muscle volume and gait speed in community-dwelling older persons: an exploratory study. *PLoS One* 2012;**7**e32829.
23. Joseph AM, Adhihetty PJ, Buford TW, Wohlgemuth SE, Lees HA, Nguyen LM *et al*. The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. *Aging Cell* 2012;**11**:801–809.
24. Calvani R, Joseph AM, Adhihetty PJ, Miccheli A, Bossola M, Leeuwenburgh C *et al*. Mitochondrial pathways in sarcopenia of aging and disuse muscle atrophy. *Biol Chem* 2013;**394**:393–414.
25. Marzetti E, Wohlgemuth SE, Lees HA, Chung HY, Giovannini S, Leeuwenburgh C. Age-related activation of mitochondrial caspase-independent apoptotic signaling in rat gastrocnemius muscle. *Mech Ageing Dev* 2008;**129**:542–549.
26. Hofer T, Marzetti E, Xu J, Seo AY, Gulec S, Knutson MD *et al*. Increased iron content and RNA oxidative damage in skeletal muscle with aging and disuse atrophy. *Exp Gerontol* 2008;**43**:563–570.
27. Altun M, Besche HC, Overkleeft HS, Piccirillo R, Edelmann MJ, Kessler BM *et al*. Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. *J Biol Chem* 2010;**285**:39597–39608.
28. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* 1997;**273**:E790–E800.
29. Marzetti E, Calvani R, Cesari M, Buford TW, Lorenzi M, Behnke BJ *et al*. Mitochondrial dysfunction and sarcopenia of aging: from signaling pathways to clinical trials. *Int J Biochem Cell Biol* 2013;**45**:2288–2301.
30. Nedergaard A, Karsdal MA, Sun S, Henriksen K. Serological muscle loss biomarkers: an overview of current concepts and future possibilities. *J Cachexia Sarcopenia Muscle* 2013;**4**:1–17.
31. Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys WG *et al*. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci* 2004;**59**:242–248.
32. Tiainen K, Hurme M, Hervonen A, Luukkaala T, Jylha M. Inflammatory markers and physical performance among nonagenarians. *J Gerontol A Biol Sci Med Sci* 2010;**65**:658–663.
33. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB *et al*. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci* 2002;**57**:M326–M332.
34. Verghese J, Holtzer R, Oh-Park M, Derby CA, Lipton RB, Wang C. Inflammatory markers and gait speed decline in older adults. *J Gerontol A Biol Sci Med Sci* 2011;**66**:1083–1089.
35. Penninx BW, Kritchevsky SB, Newman AB, Nicklas BJ, Simonsick EM, Rubin S *et al*. Inflammatory markers and incident mobility limitation in the elderly. *J Am Geriatr Soc* 2004;**52**:1105–1113.
36. Penninx BW, Pahor M, Cesari M, Corsi AM, Woodman RC, Bandinelli S *et al*. Anemia is associated with disability and decreased physical performance and muscle strength in the elderly. *J Am Geriatr Soc* 2004;**52**:719–724.
37. Visser M, Kritchevsky SB, Newman AB, Goodpaster BH, Tylavsky FA, Nevitt MC *et al*. Lower serum albumin concentration and change in muscle mass: the Health, Aging and Body Composition Study. *Am J Clin Nutr* 2005;**82**:531–537.
38. Voznesensky M, Walsh S, Dauser D, Brindisi J, Kenny AM. The association between dehydroepiandosterone and frailty in older men and women. *Age Ageing* 2009;**38**:401–406.
39. Araujo AB, Travison TG, Bhasin S, Esche GR, Williams RE, Clark RV *et al*. Association between testosterone and estradiol and age-related decline in physical function in a diverse sample of men. *J Am Geriatr Soc* 2008;**56**:2000–2008.
40. Onder G, Liperoti R, Russo A, Soldato M, Capoluongo E, Volpato S *et al*. Body mass index, free insulin-like growth factor I, and physical function among older adults: results from the ilSIRENTE study. *Am J Physiol Endocrinol Metab* 2006;**291**:E829–E834.
41. Mastaglia SR, Seijo M, Muzio D, Somoza J, Nunez M, Oliveri B. Effect of vitamin D nutritional status on muscle function and strength in healthy women aged over sixty-five years. *J Nutr Health Aging* 2011;**15**:349–354.
42. Dalal M, Ferrucci L, Sun K, Beck J, Fried LP, Semba RD. Elevated serum advanced glycation end products and poor grip strength in older community-dwelling women. *J Gerontol A Biol Sci Med Sci* 2009;**64**:132–137.
43. Semba RD, Ferrucci L, Sun K, Walston J, Varadhan R, Guralnik JM *et al*. Oxidative stress and severe walking disability among older women. *Am J Med* 2007;**120**:1084–1089.
44. Cesari M, Kritchevsky SB, Nicklas BJ, Penninx BW, Holvoet P, Koh-Banerjee P *et al*. Lipoprotein peroxidation and mobility limitation: results from the Health, Aging, and Body Composition Study. *Arch Intern Med* 2005;**165**:2148–2154.
45. Semba RD, Blaum C, Guralnik JM, Moncrief DT, Ricks MO, Fried LP. Carotenoid and vitamin E status are associated with indicators of sarcopenia among older women living in the community. *Aging Clin Exp Res* 2003;**15**:482–487.
46. Alipanah N, Varadhan R, Sun K, Ferrucci L, Fried LP, Semba RD. Low serum carotenoids are associated with a decline in walking speed in older women. *J Nutr Health Aging* 2009;**13**:170–175.
47. Marzetti E, Lorenzi M, Antocicco M, Bonassi S, Celi M, Mastropaolo S *et al*. Shorter telomeres in peripheral blood mononuclear cells from older persons with sarcopenia: results from an exploratory study. *Front Aging Neurosci* 2014;**6**:233.
48. Fragala MS, Jajtner AR, Beyer KS, Townsend JR, Emerson NS, Scanlon TC *et al*. Biomarkers of muscle quality: N-terminal propeptide of type III procollagen and C-terminal agrin fragment responses to resistance exercise training in older adults. *J Cachexia Sarcopenia Muscle* 2014;**5**:139–148.
49. Bhasin S, He EJ, Kawakubo M, Schroeder ET, Yarasheski K, Opiteck GJ *et al*. N-terminal propeptide of type III procollagen as a biomarker of anabolic response to recombinant human GH and testosterone. *J Clin Endocrinol Metab* 2009;**94**:4224–4233.
50. Chen F, Lam R, Shaywitz D, Hendrickson RC, Opiteck GJ, Wishengrad D *et al*. Evaluation of early biomarkers of muscle anabolic response to testosterone. *J Cachexia Sarcopenia Muscle* 2011;**2**:45–56.
51. Berry SD, Ramachandran VS, Cawthon PM, Gona P, McLean RR, Cupples LA *et al*. Procollagen type III N-terminal peptide (P3NP) and lean mass: a cross-sectional study. *J Frailty Aging* 2013;**2**:129–134.
52. Hettwer S, Dahinden P, Kucsera S, Farina C, Ahmed S, Fariello R *et al*. Elevated levels of a C-terminal agrin fragment identifies a new subset of sarcopenia patients. *Exp Gerontol* 2013;**48**:69–75.
53. Drey M, Sieber CC, Bauer JM, Uter W, Dahinden P, Fariello RG *et al*. C-terminal Agrin Fragment as a potential marker for sarcopenia caused by degeneration of the neuromuscular junction. *Exp Gerontol* 2013;**48**:76–80.
54. Stout JR, Fragala MS, Hoffman JR, Robinson EH, Mccormack WP, Townsend JR *et al*. C-terminal agrin fragment is inversely related to neuromuscular fatigue in older men. *Muscle Nerve* 2015;**51**:132–133.
55. Marzetti E, Calvani R, Lorenzi M, Marini F, D'Angelo E, Martone AM *et al*. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older hip fractured patients. *Exp Gerontol* 2014;**60C**:79–82.
56. Stephan A, Mateos JM, Kozlov SV, Cinelli P, Kistler AD, Hettwer S *et al*. Neurotrypsin cleaves agrin locally at the synapse. *FASEB J* 2008;**22**:1861–1873.
57. Butikofer L, Zurlinden A, Bolliger MF, Kunz B, Sonderegger P. Destabilization of the neuromuscular junction by proteolytic cleavage of agrin results in precocious sarcopenia. *FASEB J* 2011;**25**:4378–4393.
58. Ogawa K, Kim HK, Shimizu T, Abe S, Shiga Y, Calderwood SK. Plasma heat shock protein 72 as a biomarker of sarcopenia in elderly people. *Cell Stress Chaperones* 2012;**17**:349–359.
59. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW *et al*. HSP70

stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 2000;**6**:435–442.
60. Robinson MB, Tidwell JL, Gould T, Taylor AR, Newbern JM, Graves J *et al*. Extracellular heat shock protein 70: a critical component for motoneuron survival. *J Neurosci* 2005;**25**: 9735–9745.
61. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol* 2012; **8**:457–465.
62. Clark RV, Walker AC, O'Connor-Semmes RL, Leonard MS, Miller RR, Stimpson SA *et al*. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans. *J Appl Physiol (1985)* 2014;**116**: 1605–1613.
63. Stimpson SA, Leonard MS, Clifton LG, Poole JC, Turner SM, Shearer TW *et al*. Longitudinal changes in total body creatine pool size and skeletal muscle mass using the D-creatine dilution method. *J Cachexia Sarcopenia Muscle* 2013;**4**: 217–223.
64. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. *Circulation* 2007;**115**:949–952.
65. Madsen R, Lundstedt T, Trygg J. Chemometrics in metabolomics—a review in human disease diagnosis. *Anal Chim Acta* 2010;**659**:23–33.
66. Jackson JE. *A User's Guide to Principal Components*. New York: John Wiley and Sons; 1991.
67. Smilde AK, Jansen JJ, Hoefsloot HC, Lamers RJ, van der Greef J, Timmerman ME. ANOVA-simultaneous component analysis (ASCA): a new tool for analyzing designed metabolomics data. *Bioinformatics* 2005; **21**:3043–3048.
68. Bevilacqua M, Bucci R, Magrì AL, Magrì R, Nescatelli R, Marini F. Classification and class-modeling. In Marini F, ed. *Chemometrics in Food Chemistry*. Oxford: Elsevier; 2013. p171–233.
69. Szymanska E, Saccenti E, Smilde AK, Westerhuis JA. Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* 2012;**8**:3–16.
70. Marzetti E, Landi F, Marini F, Cesari M, Buford TW, Manini TM *et al*. Patterns of circulating inflammatory biomarkers in older persons with varying levels of physical performance: a Partial Least Squares-Discriminant Analysis (PLS-DA) approach. *Front Med* 2014;**1**:27.
71. Stenholm S, Tiainen K, Rantanen T, Sainio P, Heliovaara M, Impivaara O *et al*. Long-term determinants of muscle strength decline: prospective evidence from the 22-year mini-Finland follow-up survey. *J Am Geriatr Soc* 2012;**60**:77–85.
72. Sayer AA, Syddall HE, Gilbody HJ, Dennison EM, Cooper C. Does sarcopenia originate in early life? Findings from the Hertfordshire cohort study. *J Gerontol A Biol Sci Med Sci* 2004;**59**:M930–M934.
73. Kuh D, Hardy R, Butterworth S, Okell L, Richards M, Wadsworth M *et al*. Developmental origins of midlife physical performance: evidence from a British birth cohort. *Am J Epidemiol* 2006;**164**:110–121.
74. Kuh D, Hardy R, Butterworth S, Okell L, Wadsworth M, Cooper C *et al*. *J Gerontol A Biol Sci Med Sci* 2006;**61**:702–706.
75. Calvani R, Miccheli A, Landi F, Bossola M, Cesari M, Leeuwenburgh C *et al*. Current nutritional recommendations and novel dietary strategies to manage sarcopenia. *J Frailty Aging* 2013;**2**:38–53.
76. Kourti T, MacGregor JF. Process analysis, monitoring and diagnosis, using multivariate projection methods. *Chemometr Intell Lab Syst* 2014;**28**:3–21.
77. Jansen JJ, Hoefsloot HC, Boelens HF, van der Greef J, Smilde AK. Analysis of longitudinal metabolomics data. *Bioinformatics* 2004;**20**:2438–2446.
78. Eliot TS. *Collected Poems*. New York: Harcourt Brace Jovanovich; 1963.