ORIGINAL ARTICLE



# Psychometric performance of the Romanian version of the SarQoL®, a health-related quality of life questionnaire for sarcopenia

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#### Abstract

*Summary* Our study assessed the psychometric properties of the Romanian SarQoL<sup>®</sup> questionnaire. Normal distribution and high internal consistency were found. Sarcopenic subjects reported a reduced global quality of life compared to non-sarcopenics. The Romanian version of the SarQoL<sup>®</sup> questionnaire, conceptually and literally equivalent with the source instrument, is qualified in terms of psychometric properties. *Purpose/introduction* We have recently provided a translated and culturally tailored version of the first quality of life (QoL) questionnaire specific for sarcopenia, the SarQoL<sup>®</sup>, in Romanian language. The aim of this study was to assess the

psychometric performances of the translated questionnaire. *Methods* A total of 100 volunteers were enrolled in the study. Sarcopenia was diagnosed according to the algorithm proposed by the European Working Group on Sarcopenia in Older People (EWGSOP). To test the psychometric performance, discriminative power, internal consistency, floor and ceiling effects, and construct validity analyses were made. We

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assessed the correlation between SarQoL<sup>®</sup> and similar/ different domains of other two QoL questionnaires.

*Results* Sarcopenic subjects reported a reduced global QoL compared to non-sarcopenic individuals. Significantly (p = 0.018) higher total scores for non-sarcopenic subjects compared to those of sarcopenics indicate a good discriminative power of the Romanian questionnaire. Sarcopenic individuals had significantly lower scores in almost all domains. The Cronbach's alpha value of 0.946 indicates a high internal consistency. No floor or ceiling effects were found. A strong positive correlation was also found between similar domain scores from SF-36 and EQ-5D questionnaires with the Total SarQoL<sup>®</sup> score. Moreover, lower scores of quality of life have been shown to be significantly associated with lower muscle strength, in univariate analyses, and lower gait speed, both in univariate and multivariate analyses.

*Conclusions* Our results indicate that the Romanian version of the SarQoL<sup>®</sup> questionnaire, qualified in terms of psychometric properties, could be a useful tool to assess the sarcopenia-related QoL among frail Romanian individuals.

**Keywords** Sarcopenia · Quality of life · Psychometric performance · Translation · Validation

## Introduction

Sarcopenia represents an age-related, involuntary loss of skeletal muscle mass and strength. For clinical practice and research purpose, in 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) recommended the following measurable parameters for the diagnosis of sarcopenia: the muscle mass, muscle strength, and physical performance [1]. In the same way, the Foundation of the National Institute of Health Sarcopenia Project (FNIH) and the International Working Group on Sarcopenia (IWGS) have elaborated consensus definitions for sarcopenia with a specific recommendation to include two different parameters: one for muscle mass and the other for muscle function [2, 3].

The consequences of sarcopenia on QoL are difficult to evaluate and therefore are quite poorly studied. The SarcoPhAge study, conducted on a cohort developed in Liège including 534 subjects of 65 years or older, suggested that sarcopenic subjects have a significantly worse QoL in the domains of physical function compared to nonsarcopenic subjects [4]. The few studies [4, 5] assessing the QoL in sarcopenic subjects used generic QoL questionnaires, such as Short Form 36 (SF-36) and EuroQol 5dimension (EQ-5D). Generic tools do not cover exhaustively all the areas of potential dysfunction concerned in this geriatric syndrome. It would be useful to have at our disposal a sarcopenia-specific QoL questionnaire to assess not only the prospective QoL of sarcopenic subjects but also to assess the efficacy and the relevance of new therapeutic and preventive strategies developed in the field of sarcopenia [4].

SarQoL<sup>®</sup> (Sarcopenia Quality of Life) is the first diseasespecific questionnaire for sarcopenia and has been recently developed and validated initially in the French language followed a year later in English [6, 7]. The original SarQoL<sup>®</sup> is a multidimensional questionnaire designed for communitydwelling elderly subjects aged 65 years and older, consisting of 55 items, and translated into 22 questions which have been rated on a 4-point Likert scale. The 22 questions are distributed according to their relation with seven domains of dysfunction: Physical and mental health, Locomotion, Body composition, Functionality, Activities of daily living, Leisure activities, and Fears [6, 8, 9]. The translation of a standard instrument needs to be meaningful to the target population in terms of the concepts it uses and how they are expressed [10]. Cultural norms are associated with language use but not defined by them. Simply translating into another language does not ensure cultural equivalence [7].

Recently, we have provided a translated and culturally adapted version of the original SarQoL<sup>®</sup> questionnaire in Romanian language. For the translation process, we followed the source—SarQoL<sup>®</sup> authors' process as well as the international protocol of translation (forward translation to Romanian, by two independent translators, text review of the consensual version by a specialist in linguistics, a bilingual expert panel, back translation, pre-testing, cognitive interviewing, final version). The pre-test involved 20 subjects and has been described in a former published article [9].

The aim of this study was to evaluate the psychometric performances of the recently translated Romanian version of the SarQoL<sup>®</sup> questionnaire.

## Methods

#### **Study population**

The sample consisted of 100 volunteers of both sexes recruited from the Clinical County Hospital, Târgu Mureş, consecutive subjects fulfilling the inclusion criteria. These were as follows: aged 65 years old or above and a body mass index (BMI) < 30 kg/m<sup>2</sup>. Exclusion criteria included a BMI > 30 kg/m<sup>2</sup>, a history of cerebrovascular accident, heart failure, diabetes, liver cirrhosis, active tumor, patients with mental illness or inability to understand or fill the questionnaires, and other comorbidities known to have an impact on muscle mass and strength (malabsorption syndrome, Parkinson's disease, physical disabilities).

General variables analyzed are as follows: age, gender (male/female), education level (secondary school/high school/college), and physical activity (yes/no). We defined physical activity as present if the patient walked a minimum of three times per week for at least 1 h, or participates in other sport activities or farmwork in the rural area.

## Assessment of sarcopenia

Sarcopenia was defined according to the EWGSOP-proposed definition: low muscle mass and either low physical performance and/or low muscle strength.

For skeletal muscle mass estimation (SMM), we used the Lee equation:  $(0.244 \times \text{body weight}) + (7.8 \times \text{height}) + (6.6 \times \text{gender}) - (0.098 \times \text{age}) + (\text{race} - 3.3)$ ; the value 0 was used for women, 1 for men, then 0 for whites. The SMM was divided by height squared to obtain the skeletal muscle mass index (MMI). The cutoff values we used to assess skeletal muscle mass reduction were as follows:  $\leq 6.37 \text{ kg/m}^2$  for female and  $\leq 8.90 \text{ kg/m}^2$  for male patients [13, 18].

Muscle strength was assessed with a hydraulic hand dynamometer with the following cutoff values, proposed by EWGSOP, <20 kg for women and <30 kg for men. Each participant had two measurements with the dominant hand. The mean value was used.

To evaluate physical performance, we used the gait speed test: the 4-m test walk time as part of the Short Physical Performance Battery test. Patients had to walk three times and the mean value was converted to speed in meters per second (m/s) for analysis. Values under < 0.8 m/s in gait speed indicated poor physical performance.

## **Psychometric performance test**

(a) Discriminative power: The sample was divided into sarcopenic and non-sarcopenic subjects based on the criteria mentioned above. We hypothesized that QoL is better in non-sarcopenic versus sarcopenic subjects.

- (b) Internal consistency as a measure of questionnaire homogeneity was measured using the Cronbach alpha coefficient. A coefficient value greater than 0.70 indicated a high level of internal consistency (X-29). By deleting each domain at a time, its impact on the reliability was also considered.
- (c) Floor and ceiling effects were defined when a high percentage of the population had the lowest or the highest score. Floor and ceiling effects higher than 15% were considered to be significant (Y-30).
- Construct validity (convergent and divergent) analysis (d) was made only for sarcopenic subjects. Because of the small number of patients fulfilling the EWGSOP criteria for sarcopenia, modified cutoffs for the definition of sarcopenia were used: lowest sex-specific half of (appendicular) muscle mass + lowest sex-specific half of muscle strength or lowest half gait speed. With this method, 50 subjects were identified. These subjects had to complete, in addition to SarQoL®, two other questionnaires: SF-36 composed of 36 items measuring eight HRQoL domains (physical functioning, role limitation due to physical problems, pain, general health, vitality, social functioning, role limitation due to emotional problems, and mental health), scored from 0 (worst QoL) to 100 (best QoL) [14]; and also the EQ-5D questionnaire evaluating the following five dimensions: mobility, selfcare, usual activities, pain/discomfort and anxiety/depression, and a visual analog, each scored with three levels of severity [15, 16].

We assessed the correlation between SarQoL® and similar or different domains of the other two questionnaires. For the convergent validity, we used domains from the SF-36 questionnaire assumed to be similar such as D1-Physical Functioning, D2-Role limitations due to physical health, D3-Pain, D4-General Health, and D5-Vitality, in order to compare with SarQoL<sup>®</sup>-Ro total score. From the EQ-5D questionnaire, we compared dimensions of usual activities and mobility with SarQoL®-Ro. And for the divergent validity, we compared dimensions referring more to emotional health problems and the general notion of wellbeing, such as D6-Social functioning, D7-Role limitations due to emotional problems, and D8-Mental Health, from SF-36, and Self-care and Anxiety-depression, as well as Pain-discomfort, from EQ-5D.

#### Statistical analysis

All analyses described above were performed using SPSS 17.0, with a level of significance of  $\alpha = 0.05$ .

Normality of continuous variables was tested with the Shapiro-Wilk test. For population characteristics and discriminative power, quantitative variables are expressed as median (P25–P75) and qualitative variables as absolute frequencies and percentages. For continuous variables, the Mann-Whitney U test was used and, for qualitative variables, Chisquared test was used.

To measure correlation between the SarQoL<sup>®</sup> and other questionnaires, we used Pearson correlation coefficient for normality distributed data and Spearman's correlation coefficient for the non-Gaussian distributions. For internal consistency, the Cronbach alpha coefficient was used.

Moreover, we also assessed the relation between QoL and some clinical parameters such as age, sex, BMI, SMM, muscle strength, and gait speed. Correlations were measured in univariate analyses between these parameters and the SarQoL<sup>®</sup>-Ro score, both for total score and for individual domain scores. Significant parameters were then included in a linear regression to assess the relation between these parameters and QoL in multivariate analyses.

# Results

The median age of the patients was 72 (67–79) years. Gender distribution was 69% of the participants were female, and 31% male. Thirteen subjects fulfilled the criteria for sarcopenia according to the EWGSOP definition. Detailed characteristics of the subjects are presented in Table 1.

In our study group, sarcopenic subjects were significantly older and had a lower BMI compared to non-sarcopenic individuals (80 (76–88.5) vs 71 (66–77) years, p = 0.002 and 22.1 (19.8–23.1) vs 26.6 (24.8–29.1) kg/m<sup>2</sup>, p < 0.0001). We also observed in the non-sarcopenic group a larger proportion of the subjects being engaged in physical activities compared to sarcopenic individuals (p = 0.003). The education level was also higher among volunteers without sarcopenia (p < 0.001). No gender or regional differences were found between the groups.

#### **Psychometric quality analyses**

## **Discriminative power**

Sarcopenic subjects reported a reduced global QoL compared to non-sarcopenic subjects. Completed questionnaires represented significantly (p = 0.018) higher total scores for non-sarcopenic subjects compared to those with sarcopenia (68.4 (55.7–85.2) vs 57.3 (34.4–70.7)) which shows a good discriminative power of the Romanian SarQoL<sup>®</sup> questionnaire. Moreover, sarcopenic individuals have had significantly lower scores in all domains, except D4 and D6 (Functionality and Leisure Activities) (Table 2).

 Table 1
 Summary of subjects'

 general characteristics
 Image: Supplementary of Subjects and Supplementary S

Domain	N total	Sarcopenic (13)	Non-sarcopenic (87)	p value
Age (years)	100	80 (76-88.5)	71 (66–77)	0.02
Gender	100			
Female		7 (54)	62 (71)	0.21
Body mass index (kg/m <sup>2</sup> )	100	22.1 (19.8–23.1)	26.6 (24.8-29.1)	< 0.0001
Area	100			
Rural		4 (31)	64 (74)	0.81
Urban		9 (69)	23 (26)	
Physical activity	95			
Yes		8 (62)	75 (91)	0.003
Education level	100			< 0.001
Secondary school High school		6 (46) 5 (39)	6 (7) 52 (60)	
College		2 (15)	29 (33)	

#### Internal consistency

The Cronbach's alpha value of 0.946, considered "excellent," indicates a high internal consistency. When any of the domains were deleted, the alpha value remained above 0.9 and varied between 0.904 when deleting D5-Activities of daily living and 0.957—without D7-Fears. Comparing each domain with the Total SarQoL<sup>®</sup> score, we obtained positive correlation for all domains. The correlation coefficients indicate strong and very strong linear relationships between the domains and between each domain and the total score (Table 3).

#### Floor and ceiling effects

None of the subjects obtained the lowest/highest score. Therefore, no floor or ceiling effects were found for the questionnaire.

# **Convergent validity**

For the convergent validity, we compared similar domain scores from SF-36 and EQ-5D questionnaires with the Total SarQoL<sup>®</sup> score and we found a very strong positive

 
 Table 2
 Discriminative power of the SarQoL<sup>®</sup>-Ro questionnaire between different domains
 correlation between Total SarQoL<sup>®</sup> score and the domain Vitality from SF-36 (r = 0.8951, p < 0.0001). Strong correlations were found between Total SarQoL<sup>®</sup> scores with several domains from SF-36, detailed in Table 4. Regarding the EQ-5D questionnaire, we found significant but negative correlations for the following domains: Usual activities (r = 0.6106, p < 0.0001) and Mobility (r = 0.6893, p < 0.0001) (Table 4).

#### **Divergent validity**

When comparing different domains, we found a positive moderate correlation between Total SarQoL<sup>®</sup>-Ro score and D7-Role limitations, due to emotional problems from SF-36, and negative, also moderate, correlation with the domain Self-care from EQ-5D (Table 4).

#### Relation between quality and life and clinical parameters

Significant correlations have been found between QoL (total score of the SarQoL®-Ro and individual domains of QoL) and age as well as muscle strength. For age, correlations varied from  $-0.51 \ (p < 0.001) \ and -0.19 \ (p = 0.06)$  showing that the older the subjects are, the lower their QoL is. For muscle

	Sarcopenic subjects (13)	Non-sarcopenic subjects (87)	p value
Total SarQoL®-Ro score	57.3 (34.4–70.7)	68.4 (55.7–85.2)	0.018
D1-Physical and mental	52.2 (36.6–63.3)	66.6 (53.3–83.3)	0.002
D2-Locomotion	61.1 (29.2–70.8)	72.2 (55.6–91.7)	0.026
D3-Body Composition	54.2 (37.5-64.6)	66.7 (50.0–79.2)	0.014
D4-Functionality	61.5 (44.2-82.7)	75.0 (57.1–89.3)	0.10
D5-Activities of daily living	51.7 (27.5–71.7)	66.7 (48.3-85.0)	0.038
D6-Leisure activities	33.3 (33.3–58.2)	59.9 (33.3-66.5)	0.33
D7-Fears	50.0 (50.0-87.5)	87.5 (62.5–100.0)	0.046

Table 3Correlation between Total  $SarQoL^{@}$ -Ro score and each domain

	Total SarQoL <sup>®</sup> -Ro score, <i>r</i> *	p value
D1-Physical and mental	0.8890	< 0.0001
D2-Locomotion	0.9124	< 0.0001
D3-Body Composition	0.7280	< 0.0001
D4-Functionality	0.9142	< 0.0001
D5-Activities of daily living	0.9343	< 0.0001
D6-Leisure activities	0.6664	< 0.0001
D7-Fears	0.6592	< 0.0001

\*Spearman correlations (scores of the SarQoL<sup>®</sup> questionnaire normally distributed)

strength, correlations varying from 0.44 (p < 0.001) for total score and 0.24 (p = 0.01) for domain D6-Leisure activities highlighted that the higher the muscle strength is, the higher the quality of life. For the total score of the SarQoL®-Ro and domains 1 to 5, we also found significant correlations with gait speed, varying from -0.46 (p < 0.001) for total score to -0.34 (p = 0.02) for domain 3-Body composition (Table 5) showing that the more time the participants took to walk the 4-m distance, the lower their QoL.

 $\label{eq:Table 4} \begin{array}{ll} \mbox{Correlations between Total SarQoL}^{\circledast}\mbox{-Ro scores and the SF-36} \\ \mbox{and EQ-5D questionnaires} \end{array}$ 

	Total SarQoL <sup>®</sup> -Ro scores, <i>r</i>	p value
Convergent validity		
SF-36		
D1-Physical Functioning	0.8903 <sup>p</sup>	< 0.0001
D2-Role limitations due to physical health	0.6763 <sup>s</sup>	< 0.0001
D3-Pain	0.5715 <sup>p</sup>	0.0006
D4-General Health	0.6943 <sup>p</sup>	< 0.0001
D5-Vitality	0.8951 <sup>s</sup>	< 0.0001
EQ-5D		
Usual activities	-0.6106 <sup>s</sup>	0.0002
Mobility	-0.6893 <sup>s</sup>	< 0.0001
Divergent validity		
SF-36		
D6-Social functioning	0.5765 <sup>s</sup>	0.0006
D7-Role limitations due to emotional problems	0.5031 <sup>s</sup>	0.0033
D8-Mental Health	0.6822 <sup>p</sup>	< 0.0001
EQ-5D		
Self-care	-0.5356 <sup>s</sup>	0.0016
Anxiety-depression	-0.4240 s	0.0156
Pain-discomfort	-0.4580 <sup>s</sup>	0.0084

<sup>s</sup> Spearman *r* (scores normally distributed)

<sup>p</sup> Pearson *r* (scores not normally distributed)

In multivariate analysis, only gait speed still showed a significant association with the total score of QoL, once adjusted on age, sex, and muscle strength (I = -0.57; SE = 0.24; p = 0.02) (Table 6).

#### Discussion

A disease-specific tool is important to better detect the effect of treatment and observe longitudinal changes of QoL in subjects suffering from sarcopenia [5]. A current publication [11] dealing with the conduct of clinical trials in sarcopenia recommends to use patient's assessment, which is perfectly in line with the use of a QoL questionnaire [5]. This is one of the strongest rationale for developing a QoL questionnaire specific to sarcopenia. The present work aimed to offer an equivalent Romanian version of the source SarQoL<sup>®</sup> questionnaire bringing a necessary and reliable tool for assessing QoL among elderly Romanian and Moldavian patients possibly affected by sarcopenia (25 million Romanian-speaking people).

The complex transcultural adaptation resulted in a valid mean, psychometrically matched with the original version. Its high internal consistency, reliability, and construct validity confirms the measurement quality of the translated version.

In our study sample, sarcopenic subjects were significantly older and had a lower BMI compared to non-sarcopenic individuals. Interestingly, the level of education was higher among individuals with no sarcopenia. We also observed that physical activity was more present among the non-sarcopenic group (91 vs 62%). This can partly be explained by the fact that a large number of subjects in the non-sarcopenic group live in the rural area (74 vs 31%) which implies physical activity especially in the agricultural sector.

Sarcopenic subjects reported in our study a reduced global QoL compared to non-sarcopenics. For the Leisure activities domain, the discriminative power showed a difference between the two groups, but not statistically significant (33.3 (33.3–58.2) in the sarcopenic group vs 59.9 (33.3–66.5), p = 0.33). This could partly be explained by the small sample size. The data obtained are mainly consistent with French SarQoL<sup>®</sup> results. We found a good, positive, statistically significant correlation between SarQoL<sup>®</sup>-Ro total score and different SarQoL<sup>®</sup>-Ro domains.

Normal distribution and high internal consistency (convergent and divergent validity) was found. Construct validity analyses confirm significant correlations with similar dimensions of the two generic QoL questionnaires and divergence with their different domains. We found negative correlations with different domains of the EQ-5D questionnaire because higher scores in EQ-5D questionnaire mean lower QoL, hence lower SarQoL<sup>®</sup>-Ro scores.

 Table 5
 Correlations between

 the SarQoL®-Ro and clinical
 parameters

	Age (r)	BMI(r)	SMM ( <i>r</i> )	Muscle strength $(r)$	Gait speed (r)
Total SarQoL®-Ro score	-0.51*	- 0.005	0.13	0.44*	-0.46*
D1-Physical and mental	-0.41*	0.041	0.12	0.38*	-0.39*
D2-Locomotion	-0.48*	0.067	0.19	0.42*	-0.45*
D3-Body composition	-0.30*	0.022	0.17	0.25*	-0.34*
D4-Functionality	-0.41*	- 0.09	0.07	0.40*	-0.38*
D5-Activities of daily living	-0.55*	0.002	0.08	0.41*	-0.42*
D6-Leisure activities	-0.39*	-0.09	- 0.003	0.24*	-0.19
D7-Fears	-0.19	0.04	0.24*	0.33*	0.009

\*Significant Spearman correlations (scores of the SarQoL® questionnaire normally distributed)

Results also highlight the association between QoL and some clinical parameters. In univariate analyses, a lower QoL has been shown to be associated with higher age, lower muscle strength, and lower gait speed. And, this was the case not only for the total score of the SarQoL<sup>®</sup> but also for almost all individual domains of QoL. In multivariate analyses, only gait speed seemed to have a significant impact on QoL. Since the SarQoL<sup>®</sup> is a questionnaire specifically developed for subjects suffering from muscle impairments and including especially questions specific to muscle, it was expected to find significant association with grip strength, at least in univariate analyses, and gait speed.

A limitation of our study is related to the fact that we could not use DXA for muscle mass assessment. However, even so, our results qualified the translated SarQoL<sup>®</sup> questionnaire.

According to a survey completed by 255 clinicians from 55 countries across 5 continents, 66.7 (56/84) and 53.3% (24/45) of the clinicians use anthropometric measures such as calf circumference and skinfold thickness, although anthropometric measures are prone to errors and are not recommended for routine use according to EWGSOP [1, 3]. Of the clinicians, 43.3% (29/67) use absorptiometry DXA. BIA analysis as another diagnostic tool was used by 65% (13/20) of the clinicians using the single-frequency apparatus and 73% (11/15) of those using the multiple-frequency apparatus [12]. The predictive equation of Lee et al. has been proven to be a valid tool

 Table 6
 Linear regression between the total SarQoL®-Ro and clinical parameters

	Unstandardized 13	SE	Standardized 13	p value
Constant	90.89	25.1		0.001
Age	-0.49	0.31	-0.225	0.12
Sex	1.76	7.15	0.05	0.81
Muscle strength	0.52	0.35	0.32	0.14
Gait speed	-0.57	0.24	-0.33	0.02

SE standard error or regression coefficient

Model fit: R = 0.62;  $R^2 = 0.38$ ; adjusted  $R^2 = 0.32$ 

to estimate SMM. In a study published by Rech et al. [17] in an elderly population, SMM data obtained using Lee et al. equation were not statistically different from DXA-predicted SMM measurements. The mean SMM was 18.3 kg for Lee equation and 18.6 kg for DXA. The prevalence of sarcopenia between the two methods used did not differ statistically as well (36.1% for Lee equation and 33.3% for DXA).

Another limitation of this study is due to the issues related to our limited accessibility to these patients, hence the lack of a test-retest reliability evaluation. However, test-retest reliability at a 2-week interval has been shown to be excellent in the French and English validation of the SarQoL<sup>®</sup> and should therefore not be an issue. An important aspect regarding the limitations of the original SarQoL<sup>®</sup> questionnaire is the fact that the longitudinal validity is not yet known. "Sensitivity to change," considered an aspect of validity, allows the clinician using QoL tools to measure change in time [6, 7]. However, the volunteers who completed the initial SarQoL<sup>®</sup> questionnaire are part of the SarcoPhage study, and in perspective, the original developer of the questionnaire will be able to give answers on this matter [6].

Nevertheless, these results of the SarQoL<sup>®</sup> bring an additional validation among sarcopenic subjects to the SarQoL<sup>®</sup> questionnaire, which has, until now, been validated among a restricted sample of participants. The more validations will be provided, the stronger will be the evidence that this questionnaire is valuable for assessing QoL in sarcopenia.

# Conclusion

Our results indicate that the Romanian version of the SarQoL<sup>®</sup> questionnaire, conceptually and literally equivalent with the source instrument, qualified in terms of psychometric properties could be a useful tool for assessing sarcopenia-related QoL among frail, elderly individuals. This questionnaire has a greater sensitivity from this aspect compared with standard QoL questionnaires. By validating the Romanian translation, the utilization of the sarcopenia-specific QoL questionnaire, SarQoL<sup>®</sup>, will be extended and accessible to a population of 25 million (Romania and Moldavia).

**Compliance with ethical standards** All procedures 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.

#### Conflicts of interest None.

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