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Muscle, endocrine, and immunological markers of frailty in older people

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ABSTRACT

Objective: To analyze muscle, endocrine, and immunological markers that influence frailty in older people assisted in primary care.

Materials and methods: Cross-sectional, analytical, and probabilistic study were linked to the institutional research "Integrated Health Care for Older People." The study population consisted of males and females aged 60 years or more and assisted in primary health care. The research protocol included an interview and physical examination to evaluate the frailty criteria. Analysis of the following were done: serum calcium and creatinine as muscle markers; vitamin D, parathyroid hormone, and insulin-like growth factor − 1 as endocrine markers; and interleukin-6, C-reactive protein, leukocytes, and neutrophil-lymphocyte ratio as immunological markers. Statistical analysis included the Mann–Whitney test to compare means, and linear regression to analyze the relationship between dependent and independent variables.

Results: There was a relationship between creatinine and prediction of weight loss ($p < 0.001$), leukocytes and prediction of handgrip strength ($p = 0.022$), interleukin-6 and prediction of energy expenditure ($p = 0.026$), and vitamin D and prediction of gait time ($p = 0.036$). Also, sex influenced handgrip strength ($p < 0.001$), and age influenced handgrip strength ($p < 0.001$), gait time ($p < 0.001$) and energy expenditure ($p < 0.001$).

Conclusion: The joint use of muscle, endocrine, and immunological markers may be useful to diagnose frailty and to propose resolutive interventions to reduce negative outcomes for older people.

1. Introduction

Longer life expectancy and the aging population increase the occurrence of chronic non-communicable diseases, which lead to greater vulnerability, frailty, risk of morbidity, and mortality in this population (Carneiro et al., 2017). Frailty is associated with numerous negative effects in the older population, such as falls, institutionalization, hospitalization, and death; therefore, it has been the subject of studies in recent years (Alves et al., 2020). Currently, frailty is known to be affected by other factors aside from aging (Wleklik et al., 2020). Recently, frailty was considered a consequence of the contributory action of the aging process and some chronic diseases that hasten some of the changes concurrent with aging (Castellana et al., 2021).

Fried et al. defined frailty as a syndrome resulting from a spiral energy decline, especially in the muscular, endocrine, and immune systems (Fried and Walston, 2003; Fried et al., 2003). The pathophysiology of frailty shows loss of energy metabolism homeostasis through imbalanced anabolic and catabolic states in the musculoskeletal system, immune dysfunction and endocrine disruption (Wleklik et al., 2020).

As for musculoskeletal aging, there is a decrease of 3 to 4% in muscle strength and 1 to 2% in muscle mass at each passing year, and this loss is accelerated in frail people (Wleklik et al., 2020). The loss of muscle strength associated with the loss of muscle mass characterizes sarcopenia (Cruz-Jentoft et al., 2019). Some frailty-related factors, mainly reduced physical activity and physiological anorexia, aggravate anabolic insufficiency and accelerate catabolism, inducing muscle loss (Wleklik et al., 2020). Some studies show that sarcopenia can be associated with frailty (Mijnarends et al., 2015; Beaudart et al., 2015), and frail older people have a 60% increased risk of developing sarcopenia when compared to robust older people in the same age group (Mijnarends et al., 2015).

As for the immune system, the aging process causes a chronic

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inflammation called "inflammaging," which is characterized by increased pro-inflammatory cytokines in response to physiological and environmental stressors and results from the continuous cytokine production and low-grade inflammatory condition (Soysal et al., 2020). Interleukin 6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor (TNF) are inflammatory biomarkers that may be increased in the older population (Saedi et al., 2019). The accumulation of senescent cells in many tissues may be the possible biological mechanism that causes chronic inflammation (Soysal et al., 2020), and an acceleration of this inflammatory activity leads to the onset of a high-grade inflammatory condition considered a latent cause of frailty (Saedi et al., 2019).

The aging process brings several important hormonal changes that cause the functional deterioration of several physiological systems (Saedi et al., 2019). Anabolic hormones handle muscle growth and repair, so endocrine disruptions can be associated with frailty (Swiecicka et al., 2017). Many studies analyzed hormones that act on the musculoskeletal system, such as testosterone, dehydroepiandrosterone (DHEA), parathyroid hormone (PTH), vitamin D (VitD), and the insulin-like growth factor-1 (IGF-1); since the phenotypic changes in frailty are closely related with musculoskeletal changes (Saedi et al., 2019). The participation of thyroid hormones in this complex process remains unclear.

So far, the tripod of frailty has been sectioned, that is, studies show results relating frailty to only one factor proposed to cause the condition. In addition, due to the aging population worldwide and longer life expectancy, frailty has been considered as a public health priority (Bektas et al., 2018; Swiecicka et al., 2017). It was also recognized as an emerging priority by the World Health Organization (2015), and understanding its etiology is fundamental to provide early diagnosis, effective intervention, and health measures to promote active aging and prevent the onset of disabilities (Swiecicka et al., 2017). Thus, the objective of this study was to analyze muscle, endocrine, and immunological markers that influence frailty in older people assisted in primary care.

2. Materials and methods

This is a cross-sectional, analytical, and probabilistic study linked to the institutional research "Integrated Health Care for Older People"; and approved by the Human Research Ethics Committee of the Regional University of the Northwest of the State of Rio Grande do Sul under the Opinion No. 2,653,484 and CAAE: 84430917.6.0000.5350. The matrix study is a follow-up research with an execution period from 2017 to 2021 and consists of four cycles of evaluation of the subjects. The data in the present study refer to the collection in the first cycle (2017–2019).

The study population consisted of males and females aged 60 years or more who use primary health care in the urban area of a medium-sized municipality in the southern region of Brazil. The sample calculation method, sampling technique, and selection criteria were previously described by Berlezi et al. (2019). Patients with a complete physical examination protocol to evaluate frailty and laboratory tests were selected for this study.

The research protocol was done in the participants' houses and conducted in three stages: interview, physical examination, and laboratory test collection. The interview questionnaire was developed by the researchers and addressed the socio-demographic profile and general health conditions. Physical examination included the tests proposed by Fried et al. (2001) to evaluate the frailty criteria. In this study, the frailty phenotype criteria considered weight loss, handgrip strength, gait time, and energy expenditure.

The physical examination protocol was previously described (Berlezi et al., 2019). Table 1 shows the handgrip strength, gait time, and energy expenditure cutoff points established for this population. The mean cutoff points were considered in the statistical analysis to verify whether the mean values obtained were within the expected values for sex.

Laboratory tests analyzed muscle, endocrine, and immunological markers. Blood samples were collected in the reference Family Health Program or at the patient's houses and analyzed in a certified laboratory. Patients fasted for 8 h before collection and the reference values are shown in Table 2.

Table 1
Cutoff points for handgrip strength, gait time, and energy expenditure.

Sex	BMI	Cutoff points	Sex	BMI	Cutoff points
Cutoff points for handgrip strength (percentile 20)					
Male	0 < BMI < 23	20,30 kg	Female	0 < BMI < 23	13,36 kg
	23 < BMI < 28	23,52 kg		23 < BMI < 28	16,12 kg
	28 < BMI < 30	22,04 kg		28 < BMI < 30	15,17 kg
	30 < BMI < 50	25,42 kg		30 < BMI < 50	17,51 kg
Cutoff points for gait time (percentile 80)					
Sex	Height	Cutoff points	Sex	Height	Cutoff points
Male	0 < height < 1,67	≥ 7,08 s	Female	0 < height < 1,55	≥ 7,60 s
	height > 1,68	≥ 6,46 s		height > 1,56	≥ 7,45 s
Cutoff points for weekly energy expenditure (percentile 20)					
Sex	Cutoff points				
Male	1603,96 Kcal/min				
Female	2182,25 Kcal/min				

Abbreviations: BMI = body mass index; Kg = kilograms; s = seconds; Kcal/min = kilocalories per minute.
Source: Adapted from Berlezi et al. (2019).

Serum calcium and creatinine (muscle markers) were analyzed using the colorimetric method. VitD, PTH, and IGF-1 (endocrine markers) were analyzed using the electrochemiluminescence and chemiluminescence immunoassay (CLIA) methods. While IL-6 and PCR (immunological markers) were analyzed using the chemiluminescence and immunoturbidimetry methods. Leukocytes were measured from whole blood using an automated method to obtain the neutrophil-lymphocyte ratio (R/L) that serves as a predictor of chronic inflammatory process (Huguet et al., 2019).

The obtained data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 22.0. Descriptive and analytical statistical measures were defined by normal behavior using the Kolmogorov–Smirnov test. Central tendency, dispersion, and variability were used to describe quantitative variables; and relative and absolute frequencies were used to describe qualitative variables. An independent non-parametric test for samples was used to compare means (Mann–Whitney test). The linear regression model was used to analyze the relationship between dependent (weight loss, handgrip strength, gait time, and energy expenditure) and independent variables (sex and age group; and muscle, endocrine, and immunological markers). Test reliability was 95%.

3. Results

The research included 78 older people, of which 33 (42.2%) were frail and 45 (57.7%) were non-frail, 48 were women (61.5%) and 30 were men (38.5%). The mean age was 75.19 ± 8.21 years (CI95% 73.34–77.04), with 55 people aged under 80 years (70.5%) and 23 aged 80 years or more (29.5%). As for the socioeconomic data, 56 participants had a partner (71.8%) and 70 (89.7%) did not live alone, attended school, and had a family income of up to three minimum salaries.

As for the socioeconomic data in the comparison of groups, it was observed that 20 (60.6%) of the frail were 80 years old or more, while only 3 (6.7%) of the non-frail were in this age group ($p < 0.001$); 28 (62.2%) of the frail were women and 20 (60.6%) among the non-frail ($p = 0.885$). It was also found, respectively, among the frail and among the non-frail; 17 (51.5%) and 39 (86.7%) had a partner ($p = 0.001$); 27

Table 2
Cutoff points for laboratory tests.

Laboratory tests	Cutoff points	References
Muscle markers		
Calcium (mg/dL)	8,7 to 10,7	Williamson and Snyder, 2017
Creatinine (mg/dL)	Men: 0,7 to 1,6 Women: 0,6 to 1,1	Calixto-Lima and Reis, 2012
Endocrine markers		
VitD (ng/mL)	30 to 100	Williamson and Snyder, 2017
PTH (pg/mL)	12 to 65 61 to 65 years: 75 a 212 66 to 70 years: 69 a 200	Williamson and Snyder, 2017
IGF-1 (ng/mL)	71 to 75 years: 64 a 188 76 to 80 years: 59 a 177 80 years or older: 55 a 166	Williamson and Snyder, 2017
Immunological markers		
IL-6 (pg/mL)	< 3,4	Cutoff point used by the laboratory responsible for analysis (Pagana, 2015)
CRP-US (mg/dL)	< 0,3	Williamson and Snyder, 2017
Leukocytes (μL)	4.300 to 10.300	Williamson and Snyder, 2017
R/L	Men 60 to 69 years: < 2,10 70 to 79 years: < 2,25 80 to 89 years: < 2,43 90 years or older: <2,58 Women 60 to 69 years: < 1,80 70 to 79 years: < 1,95 80 to 89 years: <2,21 90 years or older: < 2,38	Huguet et al., 2019

Abbreviations: VitD = vitamin D; PTH = parathyroid hormone; IGF-1 = insulin-like growth factor-1; IL-6 = interleukin 6; CRP-US = ultrasensitive C-reactive protein; R/L = neutrophil-lymphocyte ratio.

(81.8%) and 43 (95.6%) attended school ($p = 0.065$); 28 (84.8%) and 42 (93.3%) had a family income of up to three minimum salaries ($p = 0.222$); 30 (90.9%) and 40 (88.9%) did not live alone ($p = 0.771$).

The analysis of the frailty criteria and muscle, endocrine, and immunological biochemical markers by sex and age group showed that regardless of sex, people aged 80 years or more had less muscle strength, energy expenditure, and longer gait time when compared to people aged less than 80 years (Table 3). There was a statistically significant difference for VitD, IGF-1, IL-6, and R/L in women between age groups. As for men, there was a statistically significant difference for PTH and an indication of significance for calcium, creatinine and IL-6 between age groups. The normal and changed mean values are shown in Table 3.

Table 4 shows the analysis of frailty criteria and muscle, endocrine and immunological factors according to frailty. It was observed that the frail had less handgrip strength and energy expenditure and more gait time and weight loss when compared to the non-frail. There was a statistically significant difference for calcium, IL-6 and CRP-US when comparing frail and non-frail. Still, there was a statistically significant difference for N / L. The normal and changed mean values are shown in Table 4.

Table 5 shows the simple regression model adjusted by sex and age for muscle, endocrine, and immunological markers as predictors of weight loss, handgrip strength, gait time, and energy expenditure. Statistically significant differences are also highlighted.

As for weight loss prediction, the simple model showed that older people with changed creatinine levels had a body weight decrease of 6.615 kg compared to people with normal levels. The model adjusted by sex and age group also showed that changed creatinine levels led to significantly decreased body weight (6.531 Kg).

The prediction of handgrip strength showed an increase of 10.417 kg in older men compared to women, and that people aged 80 or more had a decrease of 9.012 kg compared to people aged under 80 years. Also, older people with changed VitD, IL-6, and leukocyte levels showed a decrease of 5.976 kg, 5.922 kg, 7.176 kg, respectively compared to normal test results. The model adjusted by sex and age group showed that these markers are no longer significant, except leukocyte levels, which showed indications of significance. However, the data showed a decrease of 2.247, 3.036, and 4.223 kg in older people with changed VitD, IL-6, and leukocyte levels, respectively compared to normal test results.

The prediction of gait time showed that older people aged 80 years or more had an increase of 7.174 s compared to older people aged less than 80 years. Also, older people with changed VitD levels presented an increase of 2.709 s in gait time compared to older people with normal levels. When adjusted by sex and age group, the increase was 2.594 s, which was statistically significant. Older people with changed PTH levels showed an increase of 3.293 s in the simple model. Lastly, older people with changed IL-6 levels presented an increase of 2.657 s in gait time, but this difference was only considered as an indication of significance.

The prediction of energy expenditure showed that elderly people aged 80 or more presented a reduction of 2962.679 Kcal/min compared to people aged less than 80 years. It also showed that older people with changed IL-6 levels had a reduction of 2256.697 Kcal/min compared to older people with normal results. The adjusted model had a statistically significant difference, but the reduction was 1502.453 Kcal/min.

4. Discussion

The results of this research showed a relationship between muscle marker (creatinine) and prediction of weight loss, inflammatory markers (leukocytes and IL-6) and prediction of handgrip strength and energy expenditure, and hormonal marker (VitD) and prediction of gait time. In addition, the data shows that sex influences handgrip strength, and age influences handgrip strength, gait time and energy expenditure.

It showed that people aged 80 years or more had lower handgrip strength, longer gait time, and lower energy expenditure compared to older people aged less than 80 years, regardless of sex. Also, differences were found between mean VitD, IGF-1, IL-6, and R/L levels by age group in women, and between PTH levels by age group in men.

Age and sex are intrinsic factors that influence frailty (Carneiro et al., 2016), but these are not modifiable. There is a need for a greater understanding of frailty and its extrinsic causal factors. Therefore, it is necessary to focus on what can be modified; and the markers analyzed in this study are easy to evaluate, allowing them to be potential diagnostic and prognostic markers for frailty in older people.

Creatinine is a product of creatine phosphate degradation in muscles that represents muscle mass in people with normal kidney function; and can be used as a muscle marker (Polinder-Bos et al., 2017; Tournadre et al., 2019; Kashima et al., 2017) since direct measurement of muscle mass can be difficult in clinical situations (Kashima et al., 2017). Low muscle mass associated with low serum creatinine can be associated with weakness and weight loss, which are part of the frailty definition (Ballew et al., 2017). The prediction of weight loss seen in older people with changed serum creatinine levels in this study may be related to loss of muscle mass. Also, changed serum creatinine has also been associated

Table 3
Analysis of frailty criteria and muscle, endocrine, and immunological biochemical markers by sex and age group in older people assisted in primary care.

	Women		*p*	Men		*p*
	≥80 years	<80 years		≥80 years	<80 years	
	Mean ± SD (IC95%)	Mean ± SD (IC95%)		Mean ± SD (IC95%)	Mean ± SD (IC95%)	
Frailty criteria						
WL (Kg)	1,08[a] ± 1,80 (0,01-2,17)	0,79[a] ± 2,13 (0,04-1,55)	0,170	5,00[b] ± 9,26 (2,74-12,74)	0,71[a] ± 2,39 (0,44-1,86)	0,553
HS (Kg)	15,10[b] ± 4,67 (12,28–17,92)	21,61[a] ± 6,33 (19,36–23,85)	0,001*	20,72[b] ± 6,88 (14,96-26,47)	33,81[a] ± 9,87 (29,05-38,57)	0,001*
GT (s)	9,29[b] ± 5,98 (5,68-12,91)	4,55[a] ± 2,09 (3,81-5,29)	<0,001*	15,12[b] ± 11,44 (5,55-24,69)	3,93[a] ± 1,10 (3,40-4,46)	0,005*
EE (Kcal/min)	1193,47[b] ± 1409,21 (341,90-2045,05)	3723,90[a] ± 2163,09 (2956,91-4490,90)	<0,001*	1005,26[b] ± 827,68 (313,30-1697,23)	4798,20[a] ± 4318,41 (2716,82-6879,61)	0,001*
Muscle markers						
Calcium (mg/dL)	9,27[a] ± 0,75 (8,84-9,71)	9,34[a] ± 0,51 (9,16-9,52)	0,625	8,97[a] ± 0,51 (8,58-9,36)	9,36[a] ± 0,59 (9,09-9,63)	0,069
Creatinine (mg/dL)	0,89[a] ± 0,24 (0,75-1,02)	0,81[a] ± 0,20 (0,74 − 0,88)	0,302	1,24[a] ± 0,44 (0,90-1,58)	0,97[a] ± 0,16 (0,89-1,04)	0,081
Endocrine markers						
VitD (ng/mL)	19,16[b] ± 10,12 (13,31-25,00)	26,05[b] ± 6,52 (23,78-28,33)	0,030*	25,44[b] ± 7,82 (19,43–31,46)	30,04[a] ± 7,85 (26,47-33,62)	0,182
PTH (pg/mL)	65,80[b] ± 33,88 (46,24-85,36)	48,19[a] ± 23,21 (40,09-56,28)	0,160	56,74[a] ± 10,92 (48,34-65,15)	45,03[a] ± 31,52 (30,68-59,38)	0,011*
IGF-1 (ng/mL)	78,38[a] ± 29,90 (61,12-95,64)	102,12[a] ± 48,20 (85,30-118,93)	0,019*	102,17[a] ± 38,31 (72,72-131,61)	94,16[a] ± 25,72 (82,46-105,87)	0,839
Immunological markers						
IL-6 (pg/mL)	7,18[b] ± 7,74 (2,71-11,64)	4,40[b] ± 6,31 (2,19-6,60)	0,018*	5,34[b] ± 3,99 (2,27-8,42)	3,02[a] ± 1,85 (2,18-3,86)	0,095
CRP-US (mg/dL)	0,84[b] ± 1,35 (0,06-1,62)	0,65[b] ± 1,07 (0,28-1,03)	0,496	1,03[b] ± 1,47 (0,10-2,15)	0,39[b] ± 0,51 (0,16 − 0,62)	0,803
Leukocytes (μL)	6874,29[a] ± 2063,58 (5682,81-8065,76)	6726,76[a] ± 2346,26 (5980,12-7545,41)	0,610	6871,11[a] ± 1640,55 (5610,07-8132,15)	6228,57[a] ± 1265,63 (5652,46-6804,68)	0,230
R/L	1,92[a] ± 0,73 (1,49-2,34)	1,69[a] ± 1,46 (1,18-2,20)	0,019*	2,41[a] ± 1,28 (1,43-3,39)	1,71[a] ± 0,61 (1,43-1,98)	0,213

Abbreviations: WL = weight loss; HS = handgrip strength; GT = gait time; EE = energy expenditure; VitD = vitamin D; PTH = parathyroid hormone; IGF-1 = insulin-like growth factor-1; IL-6 = interleukin 6; CRP-US = ultrasensitive C-reactive protein; R/L = neutrophil-lymphocyte ratio.
[a] Normal values.
[b] Changed values.
* Mann-Whitney test $p < 0.05$.

with mortality (Tessier et al., 2016; Yeong-Hau, 2017), frailty, and sarcopenia (Goel et al., 2016), demonstrating the importance of monitoring this marker during the aging process.

This study also showed that immunological markers were related to frailty criteria, with leukocytes influencing handgrip strength and IL-6 influencing energy expenditure. Both inflammation and frailty increased linearly with advancing age, so the relationship between these two conditions is complex (Soysal et al., 2016). Even if it is known that the aging process has inflammatory components, further studies are needed to identify how the immune and inflammatory mechanisms regulate this process (Vatic et al., 2020). The frequent presence of comorbidities, chronic diseases, and surgical procedures at older ages are factors that stimulate the immune system; generating inflammation and, consequently, increasing white cell count and inflammatory cytokine levels (Soysal et al., 2016).

Chronic low-grade inflammation, or inflammaging, is characteristic of senescence; it triggers proteolysis and myocytic apoptosis and impairs muscle regeneration, causing skeletal muscle damages (Argilés et al., 2014). Pro-inflammatory cytokines accelerate frailty by degrading muscle proteins and, indirectly, by interfering in metabolic signaling pathways (Soysal et al., 2016). A meta-analysis conducted by Soysal et al. (2016) showed that frailty is associated with increased serum inflammatory parameters, mainly IL-6, CRP, and white cells. Castellana et al. (2021) also showed that subjects with physical frailty had higher serum levels of IL-6 and the white cells. In analyses controlled, serum levels of IL-6 were comparatively augmented among the very old participants with reduced grip strength and among those with slow walk speed (Santos Morais Junior et al., 2020). Another study associated high leukocyte counts with increased risk of sarcopenia (Chung et al., 2016). IL-6 was also proposed as a biomarker that reduces functional capacity and increases frailty, since it was negatively correlated with strength, gait speed and exercise tolerance in frail older people (Ma et al., 2018). Frailty, as well as high inflammatory levels, have a negative impact on the older population, increasing mortality, hospitalization, disability, and comorbidities (Piggott et al., 2015). In this sense, monitoring these markers is essential for the early identification of changes that may be harmful.

VitD is another modifiable and easy-to-monitor factor. An increased prevalence of VitD deficiency has been observed in the older population; this increases risk, since this vitamin plays an important role in a range of physiological processes, including the development and maintenance of the musculoskeletal system (Saedi et al., 2019) and also influencing immunity. Muscle mass and strength losses secondary to VitD deficiency are reported in the literature and may be the underlying mechanism for frailty (Wang et al., 2019). In addition, this study shows that VitD is also related to gait time, a factor that changes the functionality of older people, leading to frailty and impacts daily living activities.

Low VitD levels were associated with increased frailty rates (Saedi et al., 2019) and with factors that are predisposing to this condition (Pillatt et al., 2018). Furthermore, low VitD levels are associated with an increased risk of morbidity and mortality at all levels of frailty (Jayanama et al., 2018), and higher levels were related to reduced risks of frailty progression (Swiecicka et al., 2017). The interaction between the course of frailty and change in VitD levels is presented in the study by Van Den Berg et al. (2021), in which it was observed that an increase in VitD levels proved to be related to a decrease in continuous frailty score, that is, each frailty criterion less was related to a VitD increase of 3.04 nmol /L. In this context, oral VitD supplementation has been considered a promising approach to frailty and its consequences (Jayanama et al., 2018). Recently, a study proposed that nutrition intervention in community-dwelling older should aim at VitD levels above 75 mmol/L (30 ng/mL) (Jyväkorpi et al., 2021).

The scientific literature agrees that biochemical markers with changed levels in frailty also change during the aging process (Saedi et al., 2019), and what differentiates these conditions is the worsening of the biochemical changes. However, there are currently no reference

Table 4
Analysis of frailty criteria and muscle, endocrine, and immunological biochemical markers by frailty group in older people assisted in primary care (Brazil, 2020).

	Frail	Non-frail	*p*
	Mean ± SD (IC95%)	Mean ± SD (IC95%)	
Frailty criteria			
WL (kg)	2,64[a] ± 5,48 (0,51-4,77)	0,44[a] ± 1,64 (0,05 − 0,93)	0,009*
HS (kg)	16,50[b] ± 5,07 (14,54-18,47)	27,89[a] ± 9,31 (25,09–30,69)	<0,001*
GT (s)	10,46[b] ± 7,82 (7,43–13,50)	3,85[a] ± 1,04 (3,54-4,17)	<0,001*
EE (Kcal/min)	1123,50[b] ± 1254, 13 (637,19-1609,79)	4581,20[a] ± 3065,37 (3660,26-5502,14)	<0,001*
Muscle markers			
Calcium (mg/dL)	9,11[a] ± 0,55 (8,91-9,30)	9,42[a] ± 0,58 (9,25-9,60)	0,010*
Creatinine (mg/dL)	0,99[a] ± 0,36 (0,86-1,12)	0,86[a] ± 0,16 (0,81-0,91)	0,167
Endocrine markers			
VitD (ng/mL)	23,36[b] ± 8,97 (20,18–26,54)	27,62[b] ± 7,59 (25,34-29,91)	0,101
PTH (pg/mL)	54,30[a] ± 29,80 (43,73-64,87)	49,42[a] ± 25,68 (41,70-57,14)	0,592
IGF-1 (ng/mL)	91,78[a] ± 35,99 (79,02-104,54)	98,61[a] ± 41,81 (86,05-111,17)	0,442
Immunological markers			
IL-6 (pg/mL)	6,84[b] ± 7,38 (4,23-9,46)	3,01[a] ± 3,08 (2,09-3,94)	<0,001*
CRP-US (mg/dL)	1,07[b] ± 1,45 (0,55-1,58)	0,36[b] ± 0,49 (0,21-0,51)	0,013*
Leukocytes (μL)	6767,23[a] ± 1981,58 (6065,24-7470,52)	6538,89a ± 1959,80 (5950,10-7127,68)	0,610
R/L	1,91[a] ± 0,96 (1,58-2,26)	1,74[a] ± 1,27 (1,36-2,13)	0,085

Abbreviations: Abbreviations: WL = weight loss; HS = handgrip strength; GT = gait time; EE = energy expenditure; VitD = vitamin D; PTH = parathyroid hormone; IGF-1 = insulin-like growth factor-1; IL-6 = interleukin 6; CRP-US = ultrasensitive C-reactive protein; R/L = neutrophil-lymphocyte ratio.
[a] Normal values.
[b] Changed values.
* Mann-Whitney test $p < 0.05$.

values that can be considered predictors of frailty.

In this context, the present study proposes the analysis of markers that influence different frailty criteria and suggests that the joint use of these markers may be useful to diagnose frailty and monitor its treatment, considering that in clinical practice, the joint observation of these markers is not a common procedure. A clinical practice that considers changes resulting from senescence, supported by the analysis of muscle, immunological, and endocrine markers, leads to the proposition of a resolutive intervention in frailty and reduces the risk of outcomes such as early mortality and the onset of disabilities.

As for the limitations of the study design and methodology, the sample size is a factor that may have an influence; this may interfere with the statistical analysis of group comparison and justifies the use of indications of significance, which suggests that in larger samples the data could become significant; and the nature of the cross-sectional study, since it is not possible to verify causal factors with this methodology. It is also worth noting that this study did not analyze the presence and number of comorbidities and diseases, which can interfere with the frailty condition.

5. Conclusion

There was a relationship between creatinine and prediction of weight loss, leukocytes and prediction of handgrip strength, IL-6 and prediction of energy expenditure, and VitD and prediction of gait time. The joint use of these markers may be useful to diagnose frailty and to propose a resolutive intervention to reduce negative outcomes for older people.

Table 5
Linear regression model adjusted by sex and age for frailty criteria in older people assisted in primary care (Brazil, 2020).

Frailty criteria	Variables	Simple regression	*p*	Adjusted regression	*p*
WL (Kg)	Sex	1779	0,122	1767	0,124
	Age group	1428	0,246	1412	0,247
	Muscle markers				
	Calcium	1740	0,348	1470	0,428
	Creatinine	6615	<0,001*	6531	<0,001*
	Endocrine markers				
	VitD	-0,625	0,595	-0,320	0,794
	PTH	1827	0,178	1596	0,254
	IGF-1	1747	0,262	1796	0,244
	Immunological markers				
	IL-6	1429	0,219	1250	0,304
	CRP-US	-0,195	0,863	-0,038	0,974
	Leukocytes	-1436	0,374	-0,847	0,608
	R/L	-0,221	0,874	-0,372	0,788
HS (Kg)	Sex	10,249	<0,001*	10,417	<0,001*
	Age group	−8794	<0,001*	−9012	<0,001*
	Muscle markers				
	Calcium	−3000	0,412	−0,979	0,726
	Creatinine	−4028	0,299	−1719	0,559
	Endocrine markers				
	VitD	−5976	0,009*	−2247	0,223
	PTH	−3752	0,161	−0,827	0,695
	IGF-1	1119	0,717	2028	0,382
	Immunological markers				
	IL-6	−5922	0,009*	−3036	0,095
	CRP-US	−2147	0,336	−0,175	0,918
	Leukocytes	−7176	0,022*	−4223	0,086
	R/L	−0,570	0,836	−1219	0,558
GT (s)	Sex	1357	0,341	1259	0,289
	Age group	7193	<0,001*	7174	<0,001*
	Muscle markers				
	Calcium	0,080	0,971	−1683	0,366
	Creatinine	−0,970	0,700	−1066	0,613
	Endocrine markers				
	VitD	2709	0,055	2594	0,036*
	PTH	3293	0,045*	1422	0,320
	IGF − 1	1943	0,312	2079	0,193
	Immunological markers				
	IL-6	2657	0,065	0,727	0,569
	CRP-US	0,382	0,783	0,008	0,995
	Leukocytes	-1035	0,591	−0,527	0,751
	R/L	−1547	0,353	−1896	0,171
EE (Kcal/min)	Sex	556,399	0,439	611,737	0,344
	Age group	−2949,871	<0,001*	−2962,678	<0,001*
	Muscle markers				
	Calcium	−1316,762	0,248	−665,051	0,523
	Creatinine	−876,710	0,470	−396,976	0,718
	Endocrine markers				
	VitD	−976,861	0,179	−504,826	0,464
	PTH	−653,737	0,436	261,094	0,740
	IGF-1	−953,873	0,320	−803,029	0,354
	Immunological markers				
	IL-6	−2256,697	0,001*	−1502,453	0,026*
	CRP-US	−268,758	0,701	5725	0,993
	Leukocytes	−1253,124	0,207	−1209,490	0,189
	R/L	−277,022	0,747	−273,615	0,725

Abbreviations: WL = weight loss; HS = handgrip strength; GT = gait time; EE = energy expenditure; VitD = vitamin D; PTH = parathyroid hormone; IGF-1 = insulin-like growth factor-1; IL-6 = interleukin 6; CRP-US = ultrasensitive C-reactive protein; R/L = neutrophil-lymphocyte ratio.
* Linear regression $p < 0.05$.

CRediT authorship contribution statement

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Declaration of competing interest

None.

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