Biomarkers and the quadriceps femoris muscle architecture assessed by ultrasound in older adults with heart failure with preserved ejection fraction: a cross-sectional study

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Abstract

Background Sarcopenia is an important comorbidity in patients with heart failure with preserved ejection fraction (HFpEF). The ultrasound (US) assessment has all the advantages of being used in primary care to assess muscle quantity and quality. Some biomarkers could be indicative of muscle mass loss.

Aims To describe the quantitative and qualitative characteristics of the quadriceps femoris assessed by US in older adults with HFpEF and to assess the relationship of the blood and urinary biomarkers, the polypharmacy and comorbidities with US outcomes in older adults with HFpEF.

Methods A cross-sectional study was conducted. 76 older adults with HFpEF were included. The quadriceps femoris muscle thickness (MT, cm), the subcutaneous fat tissue thickness (FT, cm), the muscle echo intensity (MEI) and the subcutaneous fat tissue echo intensity (FEI) were assessed by US in a non-contraction (non-con) and contraction (con) situations. Polypharmacy, comorbidities, blood and urine biomarkers were also collected.

Results The carbohydrate antigen 125 (CA-125), the folic acid and the urine creatinine shared the 86.6% variance in the non-con MT, adjusted by age, sex and body mass index (BMI). The folic acid shared the 38.5% of the variance in the con MT, adjusted by age, sex and BMI. The glycosylated haemoglobin explained the 39.6% variance in the non-con MEI, adjusted by age, sex and BMI. The chlorine (Cl−) explained the 40.2% of the variance in the non-con FT, adjusted by age, sex and BMI. The polyparmacy and the folic acid explained the 37.9% of variance in the non-con FEI, while the polyparmacy and the thyrotropin (TSH) shared the 44.4% of variance in the con FEI, both adjusted by age, sex and BMI. No comorbidities, polyparmacy, or blood and urinary biomarkers could explain the con MEI and the con FT variance.

Conclusions Blood and urinary biomarkers obtained in routine analyses could help clinicians detect US outcome changes in older adults with HFpEF and identify a worsening of sarcopenia.

Trial registration NCT03909919. April 10, 2019. Retrospectively registered.

Keywords Blood biomarkers · Heart failure · Muscle thickness · Older adults · Ultrasound · Urinary biomarkers
Introduction

Heart Failure (HF) is a chronic and clinical syndrome with symptoms and/or signs caused by a structural or functional cardiac abnormality [1, 2]. Although the HF worldwide prevalence ranges from 1 to 3%, the number of patients with HF is increasing due to the population ageing [3]. It is estimated that 50% of patients with HF have a preserved ejection fraction (HFpEF) [3, 4]. Comorbidity is common in patients with HF with reduced ejection fraction (HFrEF) but slightly more severe in HFpEF [5]. Approximately 50% of patients with HFpEF have five or more major comorbidities [5]. Sarcopenia has been acknowledged as an important comorbidity in patients with HF [6, 7]. The sarcopenia prevalence is about 19.7% in patients with HFpEF [8].

Many factors related to HF potentially lead to sarcopenia, like hormonal changes, physical inactivity, oxidative stress or inflammation [9, 10]. Sarcopenia could reduce the aerobic capacity and the quality of life of patients with HFpEF [8]. Moreover, sarcopenia has also been associated with a worse prognosis in patients with HFpEF [11]. The 2018 European Working Group on Sarcopenia in Older People (EWGSOP2) recommended using low muscle strength and low muscle quantity and quality to confirm the sarcopenia diagnosis [12]. The EWGSOP2 suggested that the Skeletal Muscle Mass (SMM) or Appendicular Skeletal Muscle Mass (ASM) delimit the muscle quantity [12]. Magnetic resonance imaging (MRI) and computed tomography (CT) are gold standards for the noninvasive assessment of muscle quantity. In contrast, Dual-energy X-ray absorptiometry (DXA) is considered the reference standard [13, 14]. However, these tools are not commonly used in primary care because of high equipment costs and the lack of portability [13, 14]. Ultrasound (US) assessment has shown validity in estimating muscle quantity compared to DXA, MRI and CT [15]. The US is a portable, cheap, simple, easy-to-use, and widely available clinical practice technique that can be performed bedside and enables the physician to visualize the muscle quantity and quality with high resolution within a relatively short period [16, 17]. Thus, the US has all the advantages to be used in clinical practice and has become the standard tool for sarcopenia screening [16, 17]. US assessment has also been a reliable and valid tool to assess the muscle quantity of pennate muscles, such as the quadriceps femoris, in older adults [15]. The quadriceps femoris imaging has shown to be a good predictor of whole-body skeletal muscle mass [18, 19]. The quadriceps femoris has also become the muscle most investigated because it is easy to measure and can be linked directly to measures of physical performance [20, 21]. In this context, Kawai et al. [22] reported the possibility of using the quadriceps femoris muscle’s US quantitative and qualitative characteristics to assess muscle strength, physical function, and sarcopenia in community-dwelling older adults.

Some blood and urinary biomarkers have also been associated with sarcopenia [23–30]. Thus, urine creatinine correlated well with MRI-based measures of muscle quantity and modestly with measures from bioelectrical impedance analysis (BIA) and DXA, resulting in a good proxy measure for estimating whole-body muscle mass [26, 31]. It is unlikely that a single biomarker can identify sarcopenia because of the complex pathophysiology, so the development of a panel of biomarkers must instead be considered [27, 32, 33]. It would be important that biomarkers could detect pathophysiological mechanisms related to sarcopenia before sarcopenia clinical manifestations (reduced aerobic fitness, exercise intolerance or reduced physical performance) appear in patients with HFpEF. Blood and urinary analyses are frequent in clinical practice to assess the evolution of HF and the patient’s health status. It would be interesting for physicians to have biomarkers obtained from these routine analyses related to systemic dysfunction, which could aggravate the pathophysiological mechanisms that lead to loss of muscle quantity or quality [27]. Furthermore, these biomarkers could also monitor the effectiveness of a treatment on the pathophysiology of sarcopenia [27].

Thus, the objectives of the present study are (1) to assess the muscle thickness (MT), the subcutaneous fat tissue thickness (FT), the muscle echo intensity (MEI) and the subcutaneous fat tissue echo intensity (FEI) of the quadriceps femoris assessed by US in older adults with HFpEF and (2) to assess the relationship of the blood and urinary biomarkers, the polypharmacy and the comorbidities with these quantitative and qualitative characteristics of the quadriceps femoris in older adults with HFpEF.

Methods

Design and participants

A cross-sectional study was conducted. Seventy-six older adults with HFpEF were recruited as volunteers between April 2019 and March 2020 from the Heart Failure Unit of the Internal Medicine Service at the Regional University Hospital of Malaga (Spain). Older adults with HFpEF included in this study met the following inclusion criteria: (1) patients with HFpEF older than 70 years and diagnosed according to the European Society of Cardiology consensus statement [6]. Older adults with HFpEF were excluded if they met the following exclusion criteria: (1) older adults with HFpEF with a New York Heart Association (NYHA) class ≥ 4; (2) older adults hospitalised ≤ 3 months; (3) older adults with a score on the Mini-Mental Status Examination (MMSE) < 24.
Outcomes

US Outcomes

The right quadriceps femoris muscle and the subcutaneous fat tissue were measured in non-contraction (non-con) and contraction (con) situations. The US image was taken 15 cm from the upper edge of the patella, where transverse images were obtained with a B-mode US device (The Esaote MyLab One; Esaote, Genova, Italy) equipped with a linear array transducer 5 cm long. The transducer was placed transversely to the direction of the fibres and perpendicular to the axis of the limb. Patients were seated in a chair with their hip and knee at 90° of flexion (Fig. 1). In this same position, the patients performed a voluntary isometric contraction manually resisted by the clinician to assess the US outcomes in a con situation. Before performing the US measurements, the patients rested for 5 min to avoid bias in measuring MT and MEI muscle thickness and echo intensity [34]. The parameters used to acquire US images included the B-mode, a frequency of 10 MHz, 4 cm deep and 42% of the gain. Coupling gel was abundantly applied to minimise distortion generated by underlying tissues. Shaving was not needed. The following variables were analysed:

- **MT:** the quadriceps femoris (rectus femoris and vastus intermedius) MT was calculated using a perpendicular line to the horizontal axis from the midpoint of the femur to standardise the measurement. This line was placed between the femur and the superior fascia. The value was expressed in cm. This measurement showed a high intra-rater and inter-rater reliability, with an intraclass correlation coefficient (ICC) of 0.98 and 0.96, respectively [35]. This measurement also showed an absolute error between days of 0.017 cm [36].
- **FT:** the perpendicular line between the superior fascia and the skin. The value was also expressed in cm.
- **MEI:** the quadriceps femoris echo intensity was calculated from the selected range of interest. A histogram of the 8-bit grayscale is generated from the selected range of interest. This histogram analysed all the image pixels from 0, black, to 255, white. The average result of the resultant histogram means the MEI; that is, the MEI represents the mean pixel intensity. This outcome has no unit of measurement.
- **FEI:** the FEI was calculated the same way as the MEI. This outcome has no unit of measurement.

The combination of these variables (thickness and echo intensity) in different situations (non-con and con) and tissues (quadriceps femoris muscle and subcutaneous fat tissue) allowed obtaining eight variables: non-con MT, non-con MEI, con MT, con MEI, non-con FT, non-con FEI, con FT and con FEI.

Blood and urinary biomarkers

Blood and urine analyses performed prior to medical check-up visits collected the blood and urinary biomarkers. These analyses were performed in the hospital’s clinical laboratory. We choose blood and urine parameters which are routinely checked and do not need special analysis.

Secondary outcomes

**Clinical-epidemiological:** age, gender, educational level, marital status, number of comorbidities, medications, left ventricular ejection fraction (LVEF), NYHA class, number of falls in the last year, and smoking status.

**Anthropometric data:** weight, height, and body mass index (BMI).

Ethical issues

The study was registered on the ClinicalTrial.gov database as NCT03909919. Ethical approval was obtained from the Provincial Ethics Committee of Malaga, Spain (26.032.020). The study was carried out following the Helsinki Declaration...
femur to the superficial layer of the skin (Fig. 2). Previously, range of interest with a width of 1 cm and a height from the
for this project. This code allows the researcher to select a
US images. An own MATLAB code was created specifically
els resolution. MATLAB software (Version R2018b, Math-
US images were exported to a bmp file with 880 × 688 pix-
Sample size
The sample size was calculated using the software G Power
3.1.9.2 (University of Düsseldorf, Germany) and based on the
the following alternative hypothesis: the correlation mag-
nitude that is going to be detected between the blood and urinary biomarkers and US outcomes will be 0.6 [31] with a
significance level of 0.05 (error $\alpha < 5\%$), and statistical
power of 0.8 (80%), a sample consisting of 76 older adults
with HFpEF will be needed. The author LMP-B carried out
the recruitment in his consultations and made it possible to
obtain the estimated sample size.

Data analysis
US images were exported to a bmp file with 880×688 pix-
els resolution. MATLAB software (Version R2018b, Math-
Works, Natick, USA) was used to process and analyse the
US images. An own MATLAB code was created specifically
for this project. This code allows the researcher to select a
range of interest with a width of 1 cm and a height from the
femur to the superficial layer of the skin (Fig. 2). Previously, the researcher had to record a reference line of 1 cm, relying
on the line that shows the cm of the depth of the US image.
That 1 cm reference line formed the width of the range of
interest. Once the range of interest is selected, the code con-
verts the image to grayscale. This type of assessment showed
a high test–retest reliability score (ICC = 0.963) with an
average coefficient of variation of 4.2% [40]. Then, the oper-
ator manually also selects the superior fascia to let the code
distinguish between the muscular tissue (area between the
femur and the superior fascia) and the subcutaneous fat tis-

data to reach the sample of $n = 76$. The descriptive, anthropometric and clinical variables are shown in Sup-
plementary Appendix B. Descriptive statistics of the study
outcomes are reported in Table 1. The mean age of older
adults with HFpEF was 80.75 years old, and the mean LVEF
was 60.74. Forty-five older adults with HFpEF (59.20%) were
women, and 53 older adults showed an NYHA = 2
(69.70%). Most of the included older adults with HFpEF
showed an overweight (40.8%), and 42 older adults (55.30%)
had fallen in the past 12 months. Moreover, included older
adults showed an average of 8.41 comorbidities and took
10.18 drugs every day. The most frequent comorbidities
were arterial hypertension (97.40%), dyslipidemia (86.80%),
heart valve disease (65.80%), and chronic renal insuffi-
ciency (64.50%). Furthermore, 72 older adults with HFpEF
(94.70%) showed a left ventricular dilatation, while 41 older
adults with HFpEF (53.90%) showed left ventricular hyper-
trophy, and 37 of these older adults (48.70%) reported a left
atrial dilatation. The drugs that were most taken were loop
diuretics (85.50%), beta-blockers (73.70%) and the angioten-
sin II receptor blocker (61.80%). Older adults with HFpEF
showed a mean non-con MT of 2.06 cm (0.53) while the
con MT was 2.40 cm (0.56). The non-con FT (1.12 cm) was
larger than the con FT (1.08 cm). The non-con MEI (128.47) and the con MEI (125.61) were also larger than the non-con FEI (63.15) and the con FEI (52.22), respectively.

The bivariate correlations between the US outcomes, the muscle strength, blood biomarkers, urinary biomarkers, comorbidities, and polypharmacy are shown in Table 2. In summary, the non-con MT showed a significant but poor correlation with the polypharmacy ($r = 0.255$, $p = 0.026$), the urine creatinine ($r = 0.256$, $p = 0.030$) and the CA-125 ($r = -0.262$, $p = 0.031$). The non-con MT also showed a significant and moderate correlation with the folic acid ($r = 0.557$, $p < 0.001$). The con MT showed a significant and moderate correlation with the folic acid ($r = 0.532$, $p < 0.001$) and a significant but poor correlation with thyroid tropin (TSH) ($r = -0.247$, $p = 0.037$). The non-con MEI showed a significant but poor correlation with the glomerular filtration (GF) ($r = 0.245$, $p = 0.046$) and the glycosylated haemoglobin ($r = 0.360$, $p = 0.005$), while the con MEI did not show any correlation. The non-con FT showed a significant but poor correlation with the chlorine (Cl\(^-\)) ($r = 0.230$, $p = 0.047$). The con FT also showed a significant but poor correlation with the Cl\(^-\) ($r = 0.249$, $p = 0.032$) and the polypharmacy ($r = 0.250$, $p = 0.029$). The non-con FEI showed a significant but poor correlation with comorbidities ($r = -0.235$, $p = 0.041$), the polypharmacy ($r = -0.447$, $p < 0.01$), the Cl\(^-\) ($r = 0.236$, $p = 0.041$), the folic acid ($r = -0.294$, $p = 0.010$) and the TSH ($r = 0.246$, $p = 0.037$). The con FEI showed a significant but poor correlation with the polypharmacy ($r = -0.429$, $p < 0.01$), the Cl\(^-\) ($r = 0.280$, $p = 0.015$) and the TSH ($r = 0.348$, $p = 0.003$).

The summary of the multivariate linear regression models is shown in Table 3. Multivariate linear regression models are shown in Table 4. The CA-125, the folic acid and the urine creatinine shared 86.6% of the variance in the non-con MT, adjusted by age, sex and BMI. The folic acid shared 38.5% of the variance in the con MT, adjusted by age, sex and BMI. The glycosylated haemoglobin explained 39.6% variance in the non-con MEI, adjusted by age, sex and BMI. The Cl\(^-\) explained 40.2% variance in the non-con FT, adjusted by age, sex and BMI. The polypharmacy and the folic acid explained 37.9% variance in the non-con FEI, while the polypharmacy and the TSH shared 44.4% of the variance in the con FEI, both adjusted by age, sex and BMI. No comorbidities, polypharmacy, or blood and urinary biomarkers could explain the con MEI and the con FT variance.

**Discussion**

The present study showed the non-con MT, the con MT, non-con FT, con FT, non-con MEI, con MEI, non-con FEI and con FEI of the quadriceps femoris muscle. This study is the first study to report these US outcomes in older adults with HFpEF, apart from non-con MT, which has been reported previously. Our results reported that older adults with HFpEF have a mean non-con MT of 2.06 cm (0.53). Morimoto et al. [41] reported a median quadriceps femoris MT of 2.11 cm in patients with HFpEF and HErEF, which is similar to the results obtained by our study. Nakano et al. [42] showed a mean vastus lateralis, vastus medialis, rectus femoris and vastus intermedius MT of 5.21 cm (1.10) in patients with HFpEF and HFrEF. Nakano et al. [42] also showed that patients with HF have a reduced vastus lateralis, vastus medialis, rectus femoris, and vastus intermedius MT compared with healthy people (5.21 vs 6.54 cm). Sarcopenia is more prevalent in patients with HFpEF and HFrEF than
in healthy older adults, so it seems logical that HF patients have lower MT than healthy older adults [43]. Sarcopenia has been acknowledged as an important comorbidity in patients with HF [6, 7]. Thus, sarcopenia could reduce the aerobic capacity, the quality of life and the exercise intolerance of patients with HFrEF [8, 44]. Moreover, sarcopenia has also been associated with a worse prognosis in patients with HFrEF [11]. Muscle strength and physical functional performance are easily measurable in primary care settings [13]. On the other hand, the US has all the advantages of being used in primary care to assess muscle quantity and quality [15–17]. Therefore, it is important to collect the US outcomes assessed in our study in different age cohorts and populations, such as older adults with HF [17]. This way, pathological values can be distilled, cut-off points can be established, and correlations with the other aspects of sarcopenia-strength and function [17]. Many different mechanisms related to HF potentially lead to sarcopenia, such as hyper-activation of the sympathetic system, systemic inflammation, elevated oxidative processes, higher apoptotic activity or reduced release of the skeletal muscle [9, 10, 43]. On the other hand, sarcopenia induces impaired muscle contraction and metabolic and endocrine abnormalities that may contribute to cardiovascular remodelling and dysfunction and the development of HFrEF [45]. Previous literature has also found accentuated muscle dysfunction, with reduced mitochondrial size in skeletal muscle and increased atrophy genes and protein levels, in stable outpatients with HFrEF compared with older adults with HFrEF and healthy controls [46]. These results show the importance of muscle wasting in older adults with HFrEF, which the US could assess.

The non-con MT showed a poor correlation with the polypharmacy ($r = 0.255, p = 0.026$). The Berlin Aging Study II showed a negative relationship between appendicular lean mass and polypharmacy [47]. However, the relationship between sarcopenia and polypharmacy seems unclear because it might vary according to patients’ heterogeneous health, age, functionality, nutritional characteristics, and comorbidities [48]. The non-con MT showed a poor bivariate correlation with the CA-125 ($r = -0.262, p = 0.031$) and a moderate correlation with the folic acid ($r = 0.557, p < 0.001$). Our study is the first to report a relationship between non-con MT and CA-125 or folic acid.

It has been reported that there are higher levels of CA-125 in women with HFrEF than in healthy people [49]. A correlation between the CA-125 and the severity of systolic HF and the maximum left atrial volume has also been shown [49]. The CA-125 even could predict hospitalisation in women with HFrEF [49]. There is solid evidence of the usefulness of CA-125 as a biomarker for HF since it is easy to measure in a short period, uses widely available standardised methods, and is reasonably costly [50]. In addition, it is related to key pathophysiological processes in

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### Table 1 Descriptive statistics of the study outcomes ($n = 76$)

| US Outcomes | Mean (SD) | Min–max |
|-------------|-----------|---------|
| Thickness | | |
| Non-con MT (cm) | 2.06 (0.53) | 0.97–3.57 |
| Con MT (cm) | 2.40 (0.56) | 1.14–3.74 |
| Non-con FT (cm) | 1.12 (0.75) | 0.23–3.49 |
| Con- FT (cm) | 1.08 (0.71) | 0.23–3.28 |
| Echo intensity | | |
| Non-con MEI | 128.47 (50.61) | 45.48–254.64 |
| Con MEI | 125.61 (45.48) | 46.75–238.87 |
| Non-con FEI | 63.15 (26.13) | 16.87–137.68 |
| Con FEI | 52.22 (23.54) | 8.85–124.92 |
| Blood and urinary biomarkers | | |
| Blood biomarkers | | |
| Hb (g/dL) | 13.93 (11.11) | 6.40–108.0 |
| MCV (fL) | 95.51 (19.03) | 72–247 |
| Leukocytes count ($\times 10^9$/L) | 8.26 (4.81) | 3.54–45.20 |
| Blood platelets ($\times 10^9$/L) | 221.30 (70.98) | 8–415 |
| Glucose (mg/dL) | 102.28 (28.42) | 61–195 |
| Creatinine (mg/dL) | 1.35 (0.82) | 0.51–6.33 |
| ALT (U/L) | 22.15 (13.38) | 8–94 |
| Ferritin (ng/mL) | 123.44 (140.65) | 10–1098 |
| Glycosylated haemoglobin (%) | 6.73 (11.11) | 4.8–10.4 |
| Ferritin (mg/mL) | 213.44 (25.26) | 10–738 |
| Total cholesterol (mg/dL) | 160.76 (41.98) | 98–317 |
| LDL (mg/dL) | 86.32 (29.76) | 36–167 |
| HDL (mg/dL) | 46.78 (14.11) | 28–90 |
| Na+ (mEq/L) | 50.88 (20.42) | 8–90 |
| K+ (mEq/L) | 139.76 (3.38) | 124–148 |
| Cl− (mEq/L) | 4.64 (0.49) | 3.5–6.2 |
| CT− (mEq/L) | 101.41 (4.14) | 89–111 |
| Glycylated haemoglobin (%) | 8.70 (5.63) | 1–38 |
| Ferritin (ng/mL) | 81.42 (49.13) | 13.66–348.54 |
| Ferritin (µIU/mL) | 52.71 (83.26) | 2–525 |
| Albumin (g/dL) | 22.15 (13.38) | 8–94 |
| TSH (µIU/mL) | 2230.38 (3485.57) | 42–19,118 |
| ALT (U/L) | 52.71 (83.26) | 2–525 |
| Vitamin B12 (pg/mL) | 490.58 (642.82) | 103–4668 |
| Folic acid (ng/mL) | 221.30 (70.98) | 8–415 |
| Ferritin (ng/mL) | 221.30 (70.98) | 8–415 |
| Glycosylated haemoglobin (%) | 221.30 (70.98) | 8–415 |

SD standard deviation, US ultrasound, Non-con MT non-contraction muscle thickness, Con MT contraction muscle thickness, Non-con FT non-contraction subcutaneous fat tissue thickness, Con FT contraction subcutaneous fat tissue thickness, Non-con MEI non-contraction muscle echo intensity, Con MEI contraction muscle echo intensity, Non-con FEI non-contraction subcutaneous fat tissue echo intensity, Con FEI contraction subcutaneous fat tissue echo intensity, Hb haemoglobin, MCV mean corpuscular volume, GF glomerular filtration, Na+ sodium, K+ potassium, CI− chloride, LDL low density lipoproteins cholesterol, HDL high density lipoproteins cholesterol, NT-proBNP pro-brain natriuretic peptide, ALT alanine aminotransferase, AST aspartate transaminase, TSH thyrotropin, CA-125 carbohydrate antigen 125
HF, such as fluid overload and inflammatory activity [50]. The inflammatory activity contributes to the loss of muscle mass [51]. The CA-125 is also associated with the severity of the disease and a worse prognosis [50]. It is useful for therapeutic follow-up and could be useful to guide treatment

Table 2 Bivariate correlations ($r$) between the QFMT, the muscle strength, blood biomarkers, urinary biomarkers, comorbidities, and polypharmacy

| Comorbidities | Polypharmacy | Hb | MVC | Leukocytes Count | Blood Platelets | Glucose | Creatinine | NT-proBNP |
|---------------|--------------|----|-----|-----------------|----------------|---------|------------|-----------|
| Non-con MT    | 0.134        | 0.255* | -0.033 | 0.028          | -0.018         | 0.011   | -0.037     | -0.065    | -0.076   |
| Con MT        | 0.167        | 0.169     | -0.019 | 0.000          | -0.013         | 0.022   | -0.026     | -0.123    | -0.053   |
| Non-con MEI   | 0.007        | 0.039     | -0.118 | 0.007          | -0.011         | -0.092  | 0.210      | 0.066     | 0.146    |
| Con MEI       | -0.008       | 0.064     | -0.104 | -0.024         | 0.007          | -0.118  | 0.131      | 0.123     | 0.215    |
| Non-Con FT    | -0.070       | -0.217    | 0.192  | 0.031          | -0.095         | 0.101   | -0.110     | -0.145    | -0.103   |
| Con FT        | -0.052       | -0.250*   | 0.196  | 0.097          | -0.099         | 0.107   | -0.090     | -0.149    | -0.116   |
| Non-con FEI   | -0.235*      | -0.447**  | 0.118  | 0.048          | -0.112         | 0.061   | -0.066     | -0.118    | -0.014   |
| Con FEI       | -0.177       | -0.429**  | 0.163  | 0.131          | -0.112         | 0.018   | -0.117     | -0.085    | 0.012    |

Table 3 Summary of Models

|               | $R$  | $R^2$ | Adjusted $R^2$ | SE  | $F$   | $p$   |
|---------------|------|-------|----------------|-----|-------|-------|
| Non-con MT    | 0.931| 0.866 | 0.852          | 0.20| 61.44 | <0.001|
| Con MT        | 0.621| 0.385 | 0.350          | 0.45| 11.12 | <0.001|
| Non-con MEI   | 0.629| 0.396 | 0.351          | 0.35| 40.15 | <0.001|
| Con MEI       | 0.634| 0.402 | 0.368          | 0.60| 11.77 | <0.001|
| Non-con FT    | 0.616| 0.379 | 0.335          | 0.40| 21.31 | <0.001|
| Con FEI       | 0.666| 0.444 | 0.402          | 17.08| 10.55 | <0.001|
This study shows that CA-125 is also related to loss of muscle quantity in patients with HFpEF. Decreases in folic acid intake may also impair muscle function through their action on homocysteine. In contrast, the folic acid supplementation plus a resistance training programme could significantly increase the muscle mass in older adults [52, 53]. The non-con MT also showed a poor and directly proportional correlation with the urine creatinine \((r = 0.256, p = 0.030)\). Previous studies had reported a good correlation between the urine creatinine and the MRI, BIA and DXA measures of muscle mass being a good measure for estimating whole-body muscle mass [26, 31]. The CA-125, the folic acid and the urine creatinine shared the 86.6% variance in the non-con MT, adjusted by age, sex and BMI. Thus, these biomarkers could indicate MT loss in older adults with HFpEF.

The con MT showed a significant and moderate correlation with the folic acid \((r = 0.532, p < 0.001)\) and a significant but poor correlation with the TSH \((r = -0.247, p = 0.037)\). The TSH was also related to sarcopenia in patients with HFrEF, so it could be another important biomarker to control and monitor changes in MT [54]. Moreover, the polypharmacy, the folic acid and other blood biomarkers explained the 38.5–44.4% variance of some US outcomes reported in

### Table 4 Multivariate linear regression models

| Dependent outcome | Predictor Variables | Non-standardized coefficients | Typified coefficients | \(t\) | \(p\) | 95% CI |
|-------------------|---------------------|--------------------------------|----------------------|------|------|--------|
| **Non-con MT**    | (Constant)          | 1.477                          | 0.466                | 3.171| 0.002|(0.544, 2.410) |
| Ca-125            | -0.004              | 0.001                          | -0.420               | -7.067| 0.000|(−0.005, −0.003) |
| Folic acid        | 0.175               | 0.011                          | 1.971                | 15.947| 0.000|(0.153, 0.197) |
| Urine creatinine  | -0.016              | 0.001                          | -1.520               | -13.385| 0.000|(−0.018, −0.013) |
| Sex               | -0.103              | 0.052                          | -0.100               | -1.989| 0.051|(-0.207, 0.001) |
| Age               | 0.002               | 0.005                          | 0.027                | 0.474 | 0.637|(−0.008, 0.012) |
| BMI               | 0.012               | 0.006                          | 0.123                | 1.413 | 0.039|(−0.001, 0.024) |
| **Con MT**        | (Constant)          | 2.483                          | 0.920                | 2.699 | 0.009|(0.649, 4.317) |
| Folic acid        | 0.044               | 0.009                          | 0.470                | 4.958 | 0.000|(0.027, 0.062) |
| Sex               | -0.225              | 0.109                          | -0.197               | -2.059| 0.043|(−0.443, −0.007) |
| Age               | -0.011              | 0.010                          | -0.118               | -1.167| 0.247|(-0.031, 0.008) |
| BMI               | 0.020               | 0.010                          | 0.209                | 2.046 | 0.044|(0.001, 0.040) |
| **Non-con MEI**   | (Constant)          | 273.495                        | 103.271              | 2.648 | 0.011|(66.450, 480.541) |
| **Glycosylated Haemoglobin** | 8.860 | 4.916 | 0.201 | 1.802 | 0.077|(−0.995, 18.715) |
| Sex               | -37.003             | 11.442                         | -0.368               | -3.234| 0.002|(−59.943, −14.064) |
| Age               | -1.202              | 1.055                          | -0.130               | -1.139| 0.260|(−3.318, 0.913) |
| BMI               | -2.760              | 0.924                          | -0.344               | -2.987| 0.004|(−4.612, −0.907) |
| **Non-con FT**    | (Constant)          | -6.090                         | 1.971                | -3.090| 0.003|(−10.021, −2.159) |
| CI\(^{-}\)        | 0.044               | 0.017                          | 0.245                | 2.601 | 0.011|(0.010, 0.078) |
| Sex               | 0.429               | 0.143                          | 0.284                | 2.992 | 0.004|(0.143, 0.715) |
| Age               | 0.008               | 0.013                          | 0.060                | 0.592 | 0.556|(−0.018, 0.033) |
| BMI               | 0.062               | 0.013                          | 0.488                | 4.857 | 0.000|(0.037, 0.088) |
| **Non-con FEI**   | (Constant)          | 36.089                         | 48.014               | 0.752 | 0.545|(−59.672, 131.850) |
| Polypharmacy      | -3.418              | 0.824                          | -0.412               | -4.150| 0.000|(−5.061, −1.775) |
| Folic acid        | -1.150              | 0.420                          | -0.263               | -2.739| 0.008|(−1.987, −0.313) |
| Sex               | 12.627              | 5.150                          | 0.239                | 2.452 | 0.017|(2.357, 22.898) |
| Age               | 0.466               | 0.475                          | 0.105                | 0.981 | 0.330|(−0.481, 1.414) |
| BMI               | 0.883               | 0.464                          | 0.198                | 1.903 | 0.061|(0.042, 1.809) |
| **Con FEI**       | (Constant)          | 60.051                         | 39.544               | 1.519 | 0.134|(−18.902, 139.004) |
| Polypharmacy      | -2.808              | 0.674                          | -0.399               | -4.166| 0.000|(−4.153, −1.462) |
| TSH               | 3.679               | 1.209                          | 0.302                | 3.043 | 0.003|(1.265, 6.093) |
| Sex               | 18.035              | 4.257                          | 0.403                | 4.237 | 0.000|(9.536, 26.534) |
| Age               | 0.064               | 0.393                          | 0.017                | 0.163 | 0.871|(−0.721, 0.849) |
| BMI               | -0.174              | 0.413                          | -0.046               | -0.422| 0.674|(-0.999, 0.650) |

[50].
our study, always adjusted by age, sex and BMI. Due to the complex pathophysiology of sarcopenia, it is unlikely that there will be a single biomarker that can identify sarcopenia [32, 33]. However, the present study results reported blood and urinary biomarkers that could be related to key pathophysiological processes in muscle mass loss in patients with HFpEF. These biomarkers were obtained by routine blood and urinary analyses and could monitor treatment effectiveness [27].

Implications for clinical practice

Our results showed blood and urinary biomarkers related to pathophysiological mechanisms that lead to loss of muscle quantity and quality or to sarcopenia in patients with HFpEF. These biomarkers could allow monitoring of the effectiveness of treatment [27]. Exercise interventions can improve muscle strength, physical functional performance, and muscle mass, so older adults with HFpEF should perform resistance exercise and aerobic exercise supervised by a physical therapist to improve these outcomes [55–58]. To improve muscle mass, older adults with HFpEF should perform extended resistance exercise programmes or higher doses and intensities of resistance exercise [56, 59, 60].

Future research

Future research should assess the quadriceps femoris muscle activity by electromyography in older adults with HFpEF to assess nervous dysfunctions. Thus, an impaired electromyographic activity in older adults with HF has been reported, contributing to functional abnormalities of the skeletal muscle in the advanced stages of the HF [61]. More information should also be collected on HF and expected US abnormalities [62]. Future studies also should analyse US outcomes differences between older adults with HFpEF and older adults with HFrEF or healthy people. Future studies also could assess the effect of pharmacological and non-pharmacological treatments on these US outcomes. Kawai et al. [22] reported the possibility of using the US outcomes of the quadriceps femoris muscle to assess muscle strength, physical function, and sarcopenia in community-dwelling older adults. Thus, future studies should assess the relationship between US outcomes and muscle strength and physical functional performance in older adults with HFpEF. Moreover, future studies could confirm the findings shown by the present study, where the CA-125, the folic acid and the urine creatinine explained the 86.6% of the variance in the non-con MT, adjusted by age, sex and BMI. Future studies should also assess other biomarkers related to US outcomes.

Strengths and limitations of the study

The main strength of our study is the description of the quantitative and qualitative characteristics of the quadriceps femoris, which had not been previously analysed in older adults with HFpEF. Moreover, the present study assessed the quantitative and qualitative characteristics of the quadriceps femoris in con and non-con situations. US assessment of con MT has shown to be an objective measure superior to the assessment of relaxed muscles [63]. Another strength of the study lies in the sample size. The author IJF-A conducted all the measurements to reduce the risk of bias among sonographers [64]. The US has also shown good intra-rater reliability [35] and excellent inter-rater reliability, regardless of the sonographer’s level of experience, the severity of patient illness, or patient setting [35, 65, 66]. Thus, the IJF-A experience should not have affected the results obtained on MT. All the US measurements were performed with the same US at the same point of the quadriceps femoris and with the same US parameters. The patients were also placed in the same chair and posture to avoid biases when obtaining the US outcomes. However, several limitations must be taken into account when interpreting the results. The landmark chosen to take the images and the patient’s position could affect the interpretation and comparison of the results across studies [67]. It was reported that the transducer should be placed at 1/2 or 2/3 the distance from the anterior superior iliac spine to the patella when quadriceps femoris MT want to be assessed [42, 65, 66, 68–71]. US measurements were taken in most previous studies with the patients in the supine position with their legs and knees extended and their muscles relaxed [35, 41, 42, 65, 66, 70]. We performed the US images with the patients seated with 90º of the knee and hip flexion, and US images were taken 15 cm from the upper edge of the patella. Thus, the US image landmark and the position of the patients may have affected the muscle thickness and the correlations shown in our study. Another limitation was the imputation of the acid folic, the CA-125, the urine creatinine and the AST data, which could have caused attrition bias in the results. The morphological characteristics of the included older adults could have affected the US outcomes, causing a detection bias in these outcomes. The statistically significant and the statistically non-significant results were presented in this study to avoid publication bias.
**Conclusion**

Muscle wasting could reduce the aerobic capacity, the quality of life and the exercise intolerance of older adults with HFpEF. The US assessment could be the best tool to assess muscle quality and quantity in primary care settings. Moreover, the polypharmacy, blood and urinary biomarkers could be related to pathophysiological mechanisms of sarcopenia in older adults with HFpEF. However, future studies should confirm these findings.

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**Author contributions** AIC-V, MRB-L, RG-H and LMP-B have made a contribution to the conception of this study. LMP-B carried out the recruitment. IJF-A and MR collect the data. AIC-V, RG-H, MRB-L and LMP-B drafted the protocol. IJF-A and AIC-V participated in the analysis and interpretation of data and were involved in drafting the manuscript, as well as revising it critically for important intellectual content. All authors gave final approval of the version to be published.

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**Data availability** The data that support the findings of this study are available from the corresponding author, [AICV], upon reasonable request.

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