Identification and Characterization of New Biochemical Markers for Sarcopenia

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Passion for healthy aging

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Backgrounds

Originally described as an age-dependent loss of muscles mass, sarcopenia is currently defined as a muscle disease associated with low muscle strength as principal determinant, and low muscle quantity (mass) and quality as secondary parameters. Several methods exist to objectively measure muscle strength and mass, but globally these methods are burdensome for patients and none of these are sensitive, specific or reliable enough to diagnose and/or prognose sarcopenia.

Objectives

The objective of this study was first to identify soluble biochemical markers of sarcopenia in serum by proteomics analysis and then to characterize them for their diagnosis potential.

Methods

Patients were included and classified according to the EWGSOP definition including handgrip strength test, walking speed and dual-energy X-ray absorptiometry (DXA). Nineteen non-sarcopenic patients (control group, mean age 77.5 [min. 68 - max. 90]) and 20 sarcopenic patients (sarcopenia group, mean age 83.2 [min. 70 - max. 92]) were enrolled. As the first-line, the serum of 10 females from the sarcopenia group and of 10 females from the control group were submitted to proteomic analysis by MS/MS spectrometry to identify potential biomarkers. Perseus software was used to compare the protein expression levels between the two groups and to calculate the attached statistical significance (p-value). Biomarkers were next characterized on the entire cohort samples by Cathepsin D (CTSD) immunoassay: Western blot (not shown) & ELISA (enzyme-linked immunosorbent assay) with Abcam kit (# ab213470).

Sarcopenia diagnosis

- In 2010, the **European Working Group on Sarcopenia in Older People (EWGSOP) reached a consensus on the clinical definition and criteria for Sarcopenia diagnosis** by describing three main stages : 'pre-sarcopenia', 'sarcopenia' &'severe sarcopenia'. (Table 1)
- In 2018, the EWGSOP modified the criteria used for the diagnosis of the disease resulting in sarcopenia and severe sarcopenia cases. (Table 2)

Table 1: Sarcopenia diagnosis summary (2010 – 2018)[1]

Stage	Muscle mass		Muscle strength		Performance
Pre-sarcopenia	↓				
Sarcopenia	↓	AND	↓	OR	↓
Severe sarcopenia	↓	AND	↓	AND	↓

Main test	DXA (SMI*)	Handgrip Strength	Gait speed
Cut-Off	Men ≤ 7.25 kg/m² Women ≤ 5.67 kg/m²	Men < 30 kg Women < 20 kg	≤ 0.8 m/s

Table 2: Sarcopenia diagnosis update (2018)[2]

Stage	Muscle strength		Muscle mass		Performance
Probable sarcopenia	↓				
Sarcopenia	↓	AND	↓		
Severe sarcopenia	↓	AND	↓	AND	↓

Main test	Handgrip Strength	DXA (SMI*)	Gait speed
Cut-Off	Men < 27 kg Women < 16 kg	Men < 7.0 kg/m² Women < 6.0 kg/m²	≤ 0.8 m/s

* Skeletal Muscle Index (SMI) = ASM/height² (kg/m²);
• "Cut-off points depend upon the measurement technique" and reference study

Results

A. Proteomic Analysis

Three proteins (Aldolase A, Cathepsin D and S100A8) differentiated sarcopenic and non-sarcopenic proteome with a ratio > 1.5 when Sarcopenia is diagnosed according to EWGSOP 2010. Only one protein (Aldolase A) differentiated sarcopenic and non-sarcopenic proteome with a ratio > 1.5 when Sarcopenia is diagnosed according to EWGSOP 2018. However, the number of patients samples is smaller than in the previous analysis (Table 3).

Table 3: Comparison of serum proteome of patients classified according to EWGSOP 2010 criteria and according to EWGSOP 2018 criteria.

		Protein	Fold expression difference	P-value
EWGSOP 2010	10 controls & 10 sarcopenic	Aldolase A	4.2	**p ≤ 0.001**
		Cathepsin D	2	**p ≤ 0.001**
		S100A8	1.7	p = 0.056
EWGSOP 2018	3 controls & 6 sarcopenic	Aldolase A	2.3	**p = 0.0016**

B. Cathepsin D levels in serum measured by ELISA

Cathepsin D is a biomarker of sarcopenia according the criteria of EWGSOP 2010 (Figure 1.A). However, it is not a biomarker for sarcopenia when considering the EWGSOP 2018 for diagnosis (Figure 1.B). In this classification, sarcopenic patients have statistically lower Body Mass Index than in the control group.

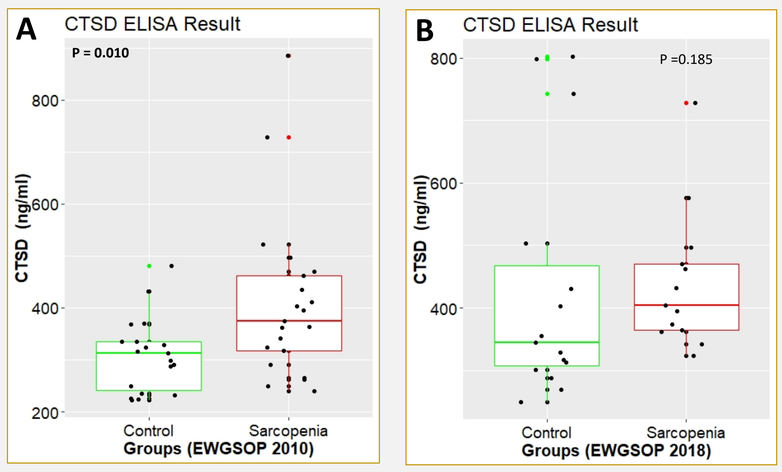


Figure 1: Cathepsin D levels **A.** between 21 sarcopenic and 19 controls according to EWGSOP 2010 and **B.** between 13 sarcopenic and 15 controls according to EWGSOP 2018

C. Cathepsin D as a biomarker for Sarcopenia (EWGSOP 2010 Criteria)

Table 4: Pearson Correlation Coefficient between CTSD and other variables following the EWGSOP 2010 classification; Number of Patients = 40

Variables	CTSD ELISA
CTSD ELISA	1.0000
6 Minute Walking Test (6MWT)	-0.3905
Tinetti_Score	-0.2983
Left Hand strength	-0.2807
Mini Nutritional Assessment (MNA) Score	-0.2737
Rigth Hand Strength Test	-0.1509
Mini-Mental State Examination (MMSE) Score	0.0998
DXA	-0.0637
Dijon Score	0.0086

Figure 2: Graph of the highest correlation (CTSD with 6MWT). 21 sarcopenic patients (*Sarco*) and 19 non-sarcopenic (*Ctrl*).

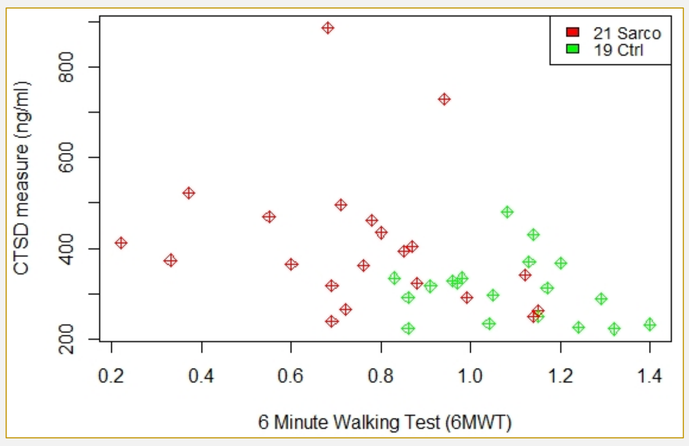
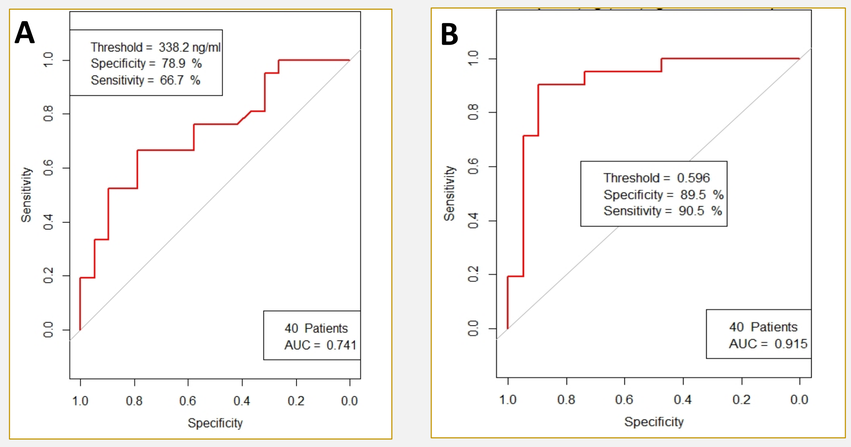


Figure 3: Receiver Operating Characteristic (ROC) curves. **A:** CTSD performance as a biomarker for sarcopenia is evaluated with a ROC curve, showing an Area Under the Curve (AUC) of 0.74. **B:** ROC curve including several variables (CTSD, Age, BMI & interaction Age*BMI) done using the values predicted by a logistic regression model shows an AUC of 0.91.



Conclusion

- **Change in the criteria used for Sarcopenia diagnosis (EWGSOP 2010 to EWGSOP 2018) had an impact on patient diagnosis and consequently on patient data distribution within these two groups.**
  Out of 58 enrolled patients, 21 were diagnosed Sarcopenic and 19 were classified as control in EWGSOP 2010.
  While out of the same group, only 13 were diagnosed as Sarcopenic and 15 as control under EWGSOP 2018.
- **Proteomic analysis of serum allows identification of promising markers**
  Biomarkers identified in the proteome using the EWGSOP 2010 are Cathepsin D, Aldolase A and S100A8 while only Aldolase A is identified using EWGSOP 2018.
  This can be the result of the lower number of patients that fit the sarcopenic and non-sarcopenic definition (loss of power in the statistical analysis)
- **Cathepsin D measured by ELISA in serum discriminates sarcopenic and non-sarcopenic patients selected using the EWGSOP 2010 criteria.**
- **Cathepsin D is correlated with physical performance test recommended by EWGSOP 2010.**

[1]A. J. Cruz-Jentoft *et al.*, « Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People », *Age Ageing*, vol. 39, nº 4, p. 412-423, juil. 2010.
[2]A. J. Cruz-Jentoft *et al.*, « Sarcopenia: revised European consensus on definition and diagnosis », *Age Ageing*, oct. 2018