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# Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older multimorbid community-dwellers: Results from the *ilSIRENTE* study

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ABSTRACT

*Background:* The C-terminal agrin fragment (CAF), a circulating byproduct of neuromuscular junction disassembly, has been proposed as a possible biomarker for sarcopenia. However, its validity in "real-world", multimorbid older persons is currently unknown. The present study was undertaken to verify if serum CAF levels were associated with sarcopenia in a population of old and very old persons living in the community.
*Methods:* Data were from the *ilSIRENTE* Aging and Longevity Study, a prospective cohort study conducted in all persons aged 80 years and older residing in the Sirente geographic area (Italy; n = 332). The identification of sarcopenia was based on the criteria elaborated by the European Working Group on Sarcopenia in Older People (EWGSOP). Serum levels of CAF were determined using a commercial ELISA kit.
*Results:* Sarcopenia was identified in 101 participants (30.8%). Serum levels of CAF were significantly higher in older adults with sarcopenia compared with non-sarcopenic participants (96.99 ± 5.40 pmol/L vs. 76.54 ± 2.15 pmol/L; $p < 0.001$). The association remained significant in both genders after adjustment for several possible confounding factors, including age, cognition, disability status, body mass index, congestive heart failure, lung diseases, diabetes, renal failure, and plasma levels of C-reactive protein and interleukin 6.
*Conclusions:* Our results obtained from a fairly large sample of old and very old, multimorbid community-dwellers show that elevated serum CAF levels are associated with sarcopenia, independent of age, gender and several clinical, functional, anthropometric, and biochemical variables. The determination of serum CAF concentration may therefore be proposed as a simple screening test for sarcopenia in the community.


## 1. Introduction

Sarcopenia, the age-related loss of muscle mass and strength or function, is a major determinant of frailty (Landi et al., 2015) and a strong predictor of several adverse health outcomes (Hirani et al., 2015). Nonetheless, the assessment of this condition is not yet part of standard practice. Indeed, the lack of a unique and standardized operational definition of sarcopenia has so far prevented healthcare professionals from developing adequate awareness on the subject (Marzetti, 2012). Another major barrier for implementing sarcopenia in standard practice resides in the limited availability of equipment for muscle mass estimation and difficulties with functional testing in frail older people, especially in community settings (Cesari et al., 2012). Magnetic resonance imaging, computed tomography and dual energy X-ray absorptiometry, although providing an objective measure of muscle or fat-free mass (Cesari et al., 2012), are not typically available in primary care (e.g., the general practitioner's office). Hence, the development and validation of biomarkers that can be measured in biofluids and used in a cost-effective manner to identify and monitor sarcopenia would mark a substantial step forward in the management of the condition (Marzetti, 2012; Calvani et al., 2015).

Denervation is invoked as one major mechanism contributing to the development and progression of sarcopenia (Kwan, 2013). Indeed, the integrity of neuromuscular junctions (NMJs) is essential for the

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maintenance of both motor nerves and muscle fibers (Shigemoto et al., 2010). NMJ remodeling has been demonstrated in old experimental animals, and linked with impairment in nerve-to-muscle signal transduction and development of sarcopenia (Oki et al., 1999). Hence, indicators of NMJ disruption could be used as biomarkers for age-related muscle wasting. The C-terminal agrin fragment (CAF) may well serve this purpose.

Agrin is a heparan sulfate proteoglycan synthesized in motor neurons, transported along axons and released into the synaptic basal lamina of NMJs. Here, it induces the assembly of the postsynaptic apparatus, including the clustering of acetylcholine receptors, and the stabilization of presynaptic structures (Stephan et al., 2008; Tezuka et al., 2014). Agrin is degraded at the NMJ by neurotrypsin to yield the 22-kDa CAF, which is released into the circulation where it can be easily measured.

In mice, overexpression of neurotrypsin in motor neurons causes NMJ disassembly, muscle atrophy and the appearance of histological features of sarcopenia (e.g., fiber size heterogeneity, centralized nuclei, fiber-type grouping, type II to type I fiber shift) (Bolliger et al., 2010; Butikofer et al., 2011). Increases in circulating CAF concentrations due to enhanced agrin cleavage may therefore be indicative of ongoing fiber denervation, which, in turn, contributes to muscle atrophy and dysfunction (Butikofer et al., 2011). Studies have indeed suggested a role for circulating CAF as a biomarker for sarcopenia (Drey et al., 2013; Hettwer et al., 2013; Fragala et al., 2014; Marzetti et al., 2014a; Stout et al., 2015). Whether serum CAF may be used as a marker for sarcopenia in "real-world", multimorbid older adults has yet to be established.

The present investigation was therefore undertaken to determine if serum concentrations of CAF were associated with sarcopenia in a fairly large population of old and very old persons living in the community, enrolled in the "Invecchiamento e Longevità nel Sirente" (Aging and Longevity in the Sirente geographic area, *ilSIRENTE*) study.

## 2. Methods

Data were from the *ilSIRENTE*, a prospective cohort study conducted in the mountain community living in the Sirente geographic area (L'Aquila, Italy). The Ethics Committee of the Catholic University of the Sacred Heart ratified the entire study protocol. All participants signed a written consent at the baseline visit. Details on the *ilSIRENTE* study protocol are described elsewhere (Landi et al., 2005).

### 2.1. Study population

A preliminary list of persons living in the Sirente area was obtained at the end of October 2003 from the Registry Offices of the 13 municipalities participating in the study. Potential participants were identified by selecting all persons born in the Sirente area before the 1st of January 1924 and living locally at the time of the survey. Among the eligible persons (n = 429), the refusal rate was very low (approximately 15%). Those who declined to participate did not differ relative to the enrollees with respect to age or gender. The overall sample enrolled in the *ilSIRENTE* study consisted of 364 participants.

The present analysis was conducted in 332 persons, after excluding 32 participants with missing data with respect to the main variables of interest.

### 2.2. Data collection

Baseline assessments of participants began in December 2003 and were completed in September 2004. The Minimum Data Set for Home Care (MDS-HC) form was administered to all study participants following the guidelines published in the MDS-HC manual (Morris et al., 1997). The MDS-HC contains over 350 data elements including socio-demographics, physical and cognitive status variables, as well as major clinical diagnoses and an extensive array of signs, symptoms, syndromes, and treatments (Morris et al., 1997). The MDS items have shown an excellent inter-rater and test–retest reliability when completed by nurses performing usual assessment duties (average weighted kappa = 0.8) (Landi et al., 2000). Additional information about lifestyle habits, physical activity and physical performance were collected using specific questionnaires and tests shared with the "Invecchiare in Chianti" (InCHIANTI) study (Ferrucci et al., 2000).

### 2.3. Assessment of sarcopenia

The identification of sarcopenia was based on the criteria elaborated by the European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft et al., 2010). Accordingly, the presence of sarcopenia was based on the documentation of low muscle mass plus either low muscle strength or low physical performance. Muscle mass was estimated from mid-arm muscle circumference (MAMC). MAMC was calculated using a standard formula (Antonelli Incalzi et al., 1996):

$$\text{MAMC} = \text{mid} - \text{arm circumference} - (3.14 \times \text{triceps skin fold thickness}).$$

The measurement of triceps skin fold thickness was obtained using a Harpenden skin fold caliper (Baty International, Burgess Hill, UK). The mid-arm circumference was determined with a standard flexible measuring tape. All measurements were taken at the right arm, unless affected by disability or diseases. In the absence of reliable cut-off points for the European population, the MAMC tertiles previously calculated in all participants enrolled in the *ilSIRENTE* study were considered (Landi et al., 2010). The lower tertile identified participants with low muscle mass. Hence, low muscle mass was defined as a MAMC smaller than 21.1 cm and 19.2 cm in men and women, respectively (Landi et al., 2010).

Habitual walking speed was evaluated by measuring the participant usual gait speed (m/s) over a 4-m course. As recommended by the EWGSOP (Cruz-Jentoft et al., 2010), a cut-off <0.8 m/s was adopted as the defining criterion for low physical performance. Muscle strength was assessed by using a North Coast hand-held hydraulic dynamometer (North Coast Medical Inc., Morgan Hill, CA). Participants performed one familiarization trial and one measurement trial with each hand, and the result from the stronger side was used for the analyses. According to the EWGSOP (Cruz-Jentoft et al., 2010), low muscle strength was defined as a handgrip strength lower than 30 kg and 20 kg in men and women, respectively.

### 2.4. Blood sampling and serum CAF determination

Fasting blood samples were obtained by venipuncture of the median cubital vein, using commercial collection tubes. Samples were processed according to standard procedures, as described elsewhere (Marzetti et al., 2014b). Plasma levels of C-reactive protein (CRP), interleukin 6 (IL6), and tumor necrosis factor alpha (TNF-α) were measured using commercially available ELISA kits on a Olympus 2700 analyzer (Olympus, Center Valley, PA).

CAF was determined in the serum through a commercial ELISA kit (NTCAF ELISA, Neurotune AG, Schlieren-Zurich, Switzerland), using a Spectramax 190 UV–VIS microplate reader (Molecular Devices, Sunnyvale, CA), as previously described (Marzetti et al., 2014a). Inflammatory biomarkers and CAF were measured in duplicate, and the average value used for the analyses. For CAF ELISA, the coefficient of variation was below 11%.

### 2.5. Covariates

Clinical diagnoses were recorded by study physicians based on information collected from the participant and his/her general practitioner, physical examination, careful review of clinical documentation (including laboratory tests and imaging exams), and previous medical