European Geriatric Medicine 7 (2016) 239–242

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Research paper

Critical analytical evaluation of promising markers for sarcopenia

E. Cavalier [a,*], C. Beaudart [b], F. Buckinx [b], O. Bruyère [b], J.-Y. Reginster [b,c]

[a] Department of Clinical Chemsitry, University of Liège, CHU Sart-Tilman, Liège, Belgium
[b] Department of Public Health, Epidemiology and Health Economics, University of Liège, Liège, Belgium
[c] Centre Académique de Recherche et d'Expérimentation en Santé SPRL (CARES SPRL), Liège, Belgium

ARTICLE INFO

Article history:
Received 14 September 2015
Accepted 4 November 2015
Available online 8 December 2015

Keywords:
Frailty
Sarcopenia
Biomarkers

ABSTRACT

We tested and validated irisin (IRI), myostatin (MYO), PIIINP, osteoglycin (OGN), TMEM119 (TMEM) and activin A (AA) and established the analytical performance, reference range and stability (considered unstable if more than 20% increase/decrease in the levels was observed in more than 10% of the samples). We were unable to obtain a valuable calibration curve with the Cusabio kits (TMEME and OGN). Coefficient of variation (CV) was too high for IRI (CV 17–30%), but were $\leq$ 10% for the 3 other analytes. AA and MYO were stable up to 3 months at −20 °C and −80 °C in serum or EDTA plasma and up to 6 months at −80 °C. PIIINP was stable only 1 month in EDTA plasma (but not in serum) at −20 °C or −80 °C. After 3 months of storage, PIIINP was not stable anymore, in serum or EDTA plasma, at −20 °C or −80 °C. Surprisingly, after 6 months at −80 °C, results returned in the ± 20% for both serum and EDTA plasma. PIIINP levels did not differ between men and women and the RR was (median, 90% CI) 1.2 (0.8–1.6)–6.0 (5.6–6.4) µg/L. The RR for MYO was 845 (437–1312)–6067 (5524–6552) pg/mL for men and 600 (268–1027)–4438 (4026–4837) pg/mL for women and the RR for AA was 177 (132–210)–622 (580–661) pg/mL for men and 98 (49–147)–480 (430–525) pg/mL for women. PIIINP and AA but not MYO accumulated in CKD as values observed in 10 hemodialyzed patients were higher than in normal individuals.

1. Introduction

Sarcopenia is a disease characterized by a loss of muscle mass and muscle function and has become a major health condition associated with ageing, which contributes to many components of public health at both the patient and the societal levels. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) has published recommendations for a clinical definition and consensual diagnosis criteria of sarcopenia [1]. According to the EWGSOP, sarcopenia is defined by the presence of low muscle mass or low muscle performance that can lead to adverse outcomes like physical disability, poor quality of life and death. Prevalence of this disease is difficult to establish and can vary according to the cut-offs points that are taken into consideration [2]. Different tools exist to assess the diagnostic of sarcopenia (recently reviewed in [3]) but the estimation of the prevalence remains linked to the diagnostic tool that has been used [4]. Contrary to many other diseases, literature is scarce on serum biomarkers that could potentially help in the diagnostic of the disease, or in the follow-up of treated or untreated patients. Hence, a biomarker could be of interest for many reasons. Among them, a biomarker is often easily obtained via a simple blood sampling, its determination is generally reproducible, can be achieved by different labs throughout the world, the levels obtained do not leave any room for subjectivity and, compared to more sophisticated techniques, it is often cheaper. However, biomarker determination is not necessarily so "simple" and several pitfalls can occur and flaw the results of a study. Among them, the precision of the assay is of course a major issue. It is indeed difficult to rely on results that have been obtained with a method presenting a coefficient of variation (CV) higher than 15%. Next to precision, two major points are often eluded in clinical studies, namely the reference ranges and the stability of the marker. Indeed, next to classical, well-established biomarkers, many emerging biomarkers used in clinical studies are generally obtained with kits for "research use only". In other words, it means that no robust reference ranges are proposed by the manufacturer and that no short or long term stability of the analyte in serum or EDTA plasma has been studied, which is of course of importance when samples are collected prospectively in clinical studies, many months before the determination of biomarkers. Finally, little is generally known on the clearance of the biomarker once in the circulation, and on its possible increase when kidney

* Corresponding author. Department of Clinical Chemistry, University Hospital of Liège, Domaine du Sart-Tilman, 4000 Liège, Belgium. Tel.: +32 4 366 7692; fax: +32 4 366 7691.
E-mail address: Etienne.cavalier@chu.ulg.ac.be (E. Cavalier).

http://dx.doi.org/10.1016/j.eurger.2015.11.002
1878-7649/

function declines. As mentioned, these important factors can totally fool the investigators of a study if they have not been established prior to sample collection. The clinical laboratory of our institution has an extensive experience in the validation and the handling of new biomarkers and, in conjunction with the clinicians that follow sarcopenic patients, we have decided to evaluate from an analytical point of view six emerging biomarkers that could potentially play a role in the management and follow-up of the patients.

2. Material and methods

2.1. Biomarkers

Six biomarkers have been selected for this validation study, namely activin A (AA) and myostatin (MYO) (R&D Systems, Abingdon, UK), procollagen III *N*-terminal peptide (PIIINP) (Orion Diagnostica, Espoo, Finland), osteoglycin (OGN) and human transmembrane protein 119 (TMEM119) (Cusabio, Wuhan, PR of China) and irisin (IRI) (Phoenix Pharmaceuticals, Karlsruhe, Germany). All of these assays were ELISA methods, except PIIIINP, which was a radio-immunoassay (RIA). The lots numbers used in this evaluation were 322439 for AA, 324636 and 322411 for MYO, 1621019 and 1635398 for PIIINP, A06076961 for OGN, Z23076960 for TMEM119 and 604944 for IRI.

These markers have been chosen because they play a role in the linkage of muscle to bone [5–7], are associated with lean mass [8] or are regulators of muscle mass [9–11].

2.2. Performance study

The precision (CV) was evaluated in accordance with a modified protocol based on CLSI EP-5A2 by running five serum samples in triplicate on five consecutive days. To obtain values spanning the dosing range, we screened for that purpose different clinical samples issued from diabetic, hemodialyzed, healthy and obese individuals.

The reference ranges were established in 120 healthy individuals (60 men and 60 non-menopause women). We evaluated the renal clearance of the markers by comparing the results obtained in the reference population and in 10 hemodialyzed patients. Finally, we studied the short-term (24 hours) and the long-term (1, 3 and 6 months) stability of the biomarkers in serum and EDTA plasma. For that purpose, we drew 5 SST tubes with gel separator and 5 tubes containing EDTA in 10 healthy volunteers. SST tubes were allowed to clot at room temperature for 30 minutes, spun 10 minutes at +4 °C and aliquoted. One fresh serum and EDTA plasma was immediately run to give the "T0" value. One aliquote of serum and EDTA plasma was determined after 24 hours of storage at +4 °C, and the others after 1, 3 and 6 months of storage at –20 °C and –80 °C. We considered that, to be reliable, the CV of an assay should be < 15%. A matrix (serum or EDTA plasma) was considered as unstable if more than 20% of the samples increased or decreased by more than 20%, compared to T0.

All the analyses have been performed in duplicates.

The characteristics of the kits, as provided by the manufacturers are presented in Table 1.

All the study was performed with the agreement of the Ethics Committee of the CHU de Liege and participants gave their informed consent.

3. Results

3.1. Analytical precision

3.1.1. Osteoglycin

The quantifiable point of the curve presented a value of 0.156 ng/mL. Unfortunately, all the human samples that we tested

Table 1
Characteristics of the different assays presented in this study, as presented by the manufacturers.

	Detection range	Sensitivity	Precision (inter-assay)	Research use only?	Reference range	Stability	Specificity	Internal QC?
AA	15.6–1000 pg/mL	From 0.75 to 7.85 pg/mL	From 4.7 to 7.9%	Yes	Human serum (n = 35): 142–753 pg/mL	Not provided	Natural and recombinant Activin A. No significant cross-reaction with different peptides tested	Yes; bought separately
MYO	31.3–2000 pg/mL	From 0.9 to 5.3 pg/mL	From 3.1 to 6%	Yes	Human serum (n = 35): 1264–8588 pg/mL	Not provided	Natural and recombinant mature myostatin. No cross-reaction with myostatin propeptide, follistatin. Recombinant human GASP-1 interferes at levels > 10 ng/mL	Yes; bought separately
PIIINP	1–50 µg/L	0.3 µg/L	From 6.5 to 7.2%	No	232 healthy adults (19–65 yo): 2.3–6.4 µg/L	5 days between 2 and 8 °C. For longer periods, store at < –20 °C	Not sensible to smaller degradation products found in blood. It measures the propeptide and its higher molecular weight form. Does not cross-react with PINP	Yes
OGN	0.156–10 ng/mL	0.039 ng/mL	< 10%	Yes	Not provided	Not provided	Osteoglycin	No
TMEM119	62.5–4000 pg/mL	< 15.6 pg/mL	< 10%	Yes	Not provided	Not provided	TMEM119	No
IRI	0.1–1000 ng/mL	Not provided	Not provided	Not provided	Not mentioned	Not provided	Not provided	Yes