

Biomarkers to Identify Protein Metabolism Impairment in Chronic/Acute Diseases

Evasio Pasini^{1*}, Roberto Aquilani², Gianni Corsetti³ and Francesco Saverio Dioguardi⁴

¹Foundation "Salvatore Maugeri", IRCCS, Medical Centre of Lumezzane (Brescia), Italy

²Foundation "Salvatore Maugeri", IRCCS, Service of Metabolic and Nutritional Pathophysiology Medical Centre of Montescano, (Pavia), Italy

³Human Anatomy and Physiopathology Unit, Department of Clinical and Experimental Science, School of Medicine, University of Brescia

⁴Department of Clinical Science and Community Health, University of Milan, Italy

Abstract

Clinical studies show that malnutrition and protein metabolic impairment in industrialised countries, is present in about 45% of elderly hospitalised patients, particularly with chronic diseases. Impairment of protein metabolism causes significant loss of body proteins including muscular proteins with sarcopenia, cachexia, morbidity and increased hospital stay and mortality. In anticipation of more sophisticated genetic and/or molecular biomarkers, here we have presented a simple rationale for the use of repeatable biomarkers that could be performed routinely at the bed-side of chronic patients. This could help clinicians to early identify and monitor protein metabolism impairment. Identifying and treating protein metabolism related abnormalities before they become irreversible and dangerous, is indispensable for the success of any therapeutic schedule.

Keywords: Biomarkers; Protein metabolism; Senescence; Chronic diseases; Acute diseases; Rehabilitation

Introduction

The maintenance of protein metabolism in humans is a fundamental condition for cell life [1]. Important organs such as the heart, liver and brain cannot survive without the integrity of protein metabolism. Interestingly, proteins are not static structures because they continuously and dynamically degrade into their basic components, i.e., amino acids (AAs) and require continuous AA support for re-synthesis. Protein (prevalently muscular) turnover in healthy subjects is about 300 g protein/day (more than 3 times normal dietary proteins intake). With normal metabolism, most AAs produced from protein breakdown are re-used for protein synthesis (up to 80%), while 20% is used to support the general metabolism. This includes energy metabolism or alternatively, they are eliminated as they are transformed into molecules that cannot be reused for syntheses (i.e., 3-methyl-histidine).

Under specific parapsychological conditions such as senescence or chronic/acute diseases such as heart failure, diabetes, COPD, stroke, sepsis and trauma, the rate of protein catabolism is high with serious impairment of protein metabolism, which correlates with morbidity and mortality [2,3].

Recent studies show that protein malnutrition and hyper-catabolic states are often found in chronic disease and are more pronounced in elderly patients (>65 years), who nowadays, make up the majority of hospitalised patients. Both past and recent data show that protein metabolic impairment are present in about 45% of elderly patients hospitalised from a variety of chronic diseases and in peculiar in chronic heart failure patients [2].

Data from recent research suggest that early identification and evaluation of protein metabolic impairment is a fundamental step for better patient care, avoiding additional and independent damage and allowing traditional therapy to work properly. As a result, assessment by specific biomarkers of early changes in protein metabolism may be fundamental to identify and monitor initial damage and protein metabolic-related abnormalities before they are irreversible [4].

Biomarkers of protein metabolism impairment

We believe that a single ideal biomarker of protein metabolism impairment does not exist. Indeed, each has certain advantages, disadvantages and/or limits as we have shown here. However, we recommend that critical evaluation of protein impairment and patients' clinical status needs to be integrated for better patient care.

Evaluation of Body Mass Index (BMI) should be the first step to assess metabolic impairment of patients as 85% of ideal body weight is an index of protein-calorie malnutrition. However, BMI is not always foolproof to detect protein impairment because it cannot distinguish lean muscular from fatty tissues separately. In addition, BMI is also influenced by fluid retention, which is a common finding in chronic disease such as CHF, or severe liver insufficiency with ascites. Weight loss (by eliminating excess water), in these patients, is a consequence of successful therapy. Body global protein metabolism can be more reliably measured by static, functional and dynamic biomarkers.

Static biomarkers

Anthropometric measurements: These allow clinicians to distinguish muscular lean mass from fatty mass.

Visceral blood proteins such as: albumin, transferrin, pre-albumin, retinol binding protein. They provide information on visceral protein synthesis.

Anthropometrical measurements: Body anthropometrical

***Corresponding author:** Evasio Pasini, Foundation "Salvatore Maugeri", IRCCS, Medical Centre of Lumezzane (Brescia), Italy, Tel: +39-0308253011; E-mail: evpasini@gmail.com

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measurements have been described in detail in the Anthropometric Indicator Measurements Guide by Food and Nutritional Technical Assistance Project Academy and elsewhere [5,6].

Most anthropometrical measurements of body composition can be performed with Dual-Energy X-Ray Absorptiometry (DEXA or DXA). However, DXA scanners are expensive equipment, which also require specific medical skill to manage and interpret. In addition, although DXA uses low energy X-ray, patients are still exposed to radiation.

Skin fold Thickness and Arm Muscle Area are simpler methods to analyse body composition and indirectly evaluate general protein metabolism. Importantly, these biomarkers can be routinely evaluated at the patient's bedside so specific training is not needed. Although these measures may indicate muscular wasting with reduced muscular proteins they do not give any suggestions about the underlying molecular mechanism.

Skin fold thickness is an index of fatty mass. It should be measured by plicometer in specific body sites such as:

- **Triceps (TSF):** Measuring along the midline on the back of the triceps of the right arm pinching the skin so that the fold is running vertically. This measurement is the easiest and simplest to collect and it is most commonly used.
- **Pectoral:** Using a line from the fold of the axillary to the nipple, determining the midpoint,
- **Abdominal:** Measuring about 1 inch laterally to the right side, from 0.5 inch below the umbilicus lifting a horizontal fold of skin,
- **Suprailiac:** Measuring the top of the iliac crest,
- **Thigh:** Using a midline of the front of the thigh and measuring midway between the inguinal crest.

Skin fold thickness is measured using a calliper by lifting the skin up from the muscle and waiting four seconds before reading the plicometer as the fat is compressible and measurements before or after four seconds may affected the results.

Arm Muscle Area (AMA) is an index of lean muscular mass.

First, we have to measure the Mid-Arm Muscle Circumference (MAMC) in centimetres, from the mid points of the left upper arm, straightening the patient's arm and wrapping the tape around the arm at its midpoint, making sure that the tape is tight and reading the measurement.

Then, the AMA can be calculated also using TSF according to the following formula:

$MAMC (cm) = MAC (cm) - 3.14 \times TSF (mm) / 10 - [(3.14 \times TSF (mm) / 10)]$. TSF and AMA are not influenced by excessive extra-cellular fluids and can be used in patients with fluid retention. Table 1 shows data corresponding to the 5th percentile of AMA found in subjects according to age and gender [7].

Blood visceral proteins assessment

Albumin, transferrin, pre-albumin (also known as Trans-Thyretin = TTR), Retinol Binding Proteins (RBP), are the most commonly used serum proteins. They have different blood half-lives with different turnovers. Therefore, they can be used to evaluate both protein metabolism and should be used to monitor the effects of therapeutic

intervention. However, these biomarker should be used with care in oedematous patients because excessive extra-cellular fluid influences serum protein concentrations [8].

Albumin: Serum level of albumin <3.5 g/l is considered a biomarker of significant reduced protein metabolism. Concentrations less than 3.2 g/l should be considered a biomarker of severe impairment, possibly associated with the onset of cachexia. In addition, it is easy to perform and is readily available. Notably, albumin blood concentration correlates with morbidity and mortality. However albumin has the blood half-life of about 20 days, consequently it is not a rapid biomarker of successful protein synthesis after nutritional intervention.

Moreover, severe nephrosis, protein-losing enteropathy, very severe liver insufficiency and fluid retention can limit the usefulness of albumin measurement.

Transferrin: This is a beta-globulin which transports iron in the blood. It's correlate with a mortality risk in hospitalized patients so it is also a good index to predict patient prognosis.

It has a blood half-life of about 8 days and this makes transferrin useful also for monitoring the medium-term effects of specific nutritional therapies.

However, transferrin blood concentration is significantly influenced by iron blood availability and infection, consequently, sideremia and inflammation should be measured to evaluate the meaning of blood transferrin concentrations.

Pre albumin (TTR): Pre albumin is involved in thyroxine transport and as a carrier for retinol. It has a blood health-life of only about 24-36 hours, so it responds quickly to changes in protein metabolism. Consequently, repeated measurements of pre-albumin are useful to measure both protein depletion due to worsening of the metabolism, and/or repletion after specific therapy. Notably, a pre albumin measurement is not reliable with renal failure (due to continuous losses) and acute metabolic stress such as acute infection. In addition, it is performed only in more specialised laboratories.

Retinol binding protein: This is a carrier for retinol. It has a turnover of about 12 hours. Therefore, it is used to monitor rapid changes of protein metabolism. It is widely used in Intensive Care Units, but it is less commonly available in less specialized laboratories and it is not considered any more useful than pre-albumin evaluation (Table 2).

Functional biomarker

Lymphocyte blood count is an indirect functional biomarker of cell proliferation, protein synthesis and energy availability. Reduced lymphocyte protein metabolism causes cell-cycle loss with consequent low counts of circulating lymphocytes. Scarce circulating lymphocytes induce malfunction of systemic immunity with increased risk of

Age	5 th Percentile
18-29.9	34.2-36.6
30-39.9	37.9-38.5
40-49.9	38.4-37.7
50-59.9	36.0-36.5
60.0-69.9	34.5-31.4
70-74.9	29.7

Table 1: 5th Percentile of upper - arm muscle area (cm²) by age, for male and female patients of 18 to 75 years.

-	BMI <20,
-	Weigh loss >10% over the last 6 months,
-	TSF <3 mm,
-	<5 th percentile of normal Upper-Arm Muscle Area (cm ²)
-	Albumin: <3.5 g/d in sarcopenia; <3.2 g/dl in cachexia
-	transferrin <180 mg/dl;
-	prealbumin 15-12 mg/dl (mild impairment), 8-10 mg/dl (moderate impairment), <8 mg/dl (severe impairment)
-	Retinol Binding Protein <2.6 mg/dl;
-	Blood Lymphocyte from 1200 to 800 cells/mm ³ (moderate impairment). <800 cells/mm ³ (severe impairment)
-	Blood 3 Met-I >4 microMol/L

Table 2: Clinical and laboratory Biomarkers of Protein Metabolism Impairment.

infection. This condition creates a vicious circle which can influence the evolution of chronic diseases [9].

We previously demonstrated that a reduction >20% of lymphocytes in 423 patients with chronic disease as Chronic Heart Failure (CHF) followed-up for 3 years, significantly increases all causes of mortality including infection [10]. Recent data have confirmed these findings. The Seattle Heart Failure Model by univariate analysis shows that white blood cell counts are associated with increased mortality in CHF patients [11].

This biomarker it is easy to monitor but it should be interpreted together with other information. For example, it may be low in cases of important impairment of protein metabolism.

Dynamic biomarkers

Nitrogen balance (NB): is the ratio of nitrogen introduced into our organism from consumed food AAs and the quantity of nitrogen excreted from the urine. Basically, NB is considered the difference between the proteins introduced and used for syntheses (anabolic processes), and those demolished in the same period (catabolic processes). In the catabolic process, the nitrogen group is removed from the carbon skeleton of AAs by a reaction called transamination, then carbon skeleton of amino acids is used to produce energy in Krebs's cycle or used as a precursor for either gluconeogenesis or lipid synthesis. As a consequence, AAs are not used for protein synthesis but rather to temporary support global body metabolism.

NB is expressed as g/day according to the formula:

Nitrogen Intake (N_i) – Nitrogen Output by urine (N_v) + 2 g where:

- N_i = nitrogen intake/supply in g/day evaluated by protein intake (g/day) where g Nitrogen is equal to intact protein/6.25 g. Intake is quantified as food eaten shown on a three-day diary. Importantly, the type and weight of cooked and uncooked food before and after meals should be recorded. When necessary, these data are converted into raw equivalents using an appropriate table.
- N_v = urinary nitrogen excretion as urea in g/day + 20% N_v for non-urea N excretion
- 2 g is the nitrogen lost in faeces and sweat.

We know that NB is in equilibrium if ± 1 g/day. NB>+1 g/day indicates the prevalence of protein synthesis. NB<-1 g/day suggests the prevalence of protein degradation with AA overwhelmingly used for general metabolic purposes instead of protein synthesis. So, NB<1 g/day suggests impairment of protein metabolism [12].

However, NB is not easy to obtain or calculate. We have to measure

both daily food intake including protein using a food diary and urea concentrations in 24 hour period of urine. Consequently, this can be performed principally only with hospitalised patients. Moreover, NB depends mostly on the urea concentrations excreted and this in turn, depends on urea synthesis. As recently indicated, urea concentrations in plasma and urines critically depend on the arginine content of diets, because arginine is the precursor of urea synthesis, cleaved by arginases and forming urea and ornithine mostly, but not exclusively, by the liver. Arginase 1 (in liver and erythrocytes) is rapidly inducible by an elevate arginine content in diets. These modifications have multiple consequences; the misleading achievement of sufficient essential amino acids intake by a positive NB due to rapid elevation of plasma and urinary urea is just a part of this very complex clinical picture [13].

3-Methyl histidine (3-MeH): is an index of muscular proteolysis with resulting AAs release. 3-MeH originates from methylation of muscular histidine. This molecule is released from the muscular cells because 3-MeH cannot be re-used for protein synthesis or metabolised elsewhere, so it directly correlates with muscular protein turnover. This process is stimulated by catabolic hormones and/or insufficient qualitative and quantitative nutrient intake including AAs. [14,15]. Therefore, massive presence of 3-MeH in the blood or urine would suggest muscular protein degradation. On the contrary, reduced circulating and/or urinary 3-MeH after therapeutic interventions would suggest that muscle proteolysis has decreased and the skeletal muscle mass and protein metabolism are preserved.

3-MeH is not easy to perform and needs specific equipment, In addition, it only gives information about muscular protein breakdown and its value should be integrated with other biomarkers and indicators.

Conclusions

Before sophisticated genetic and/or molecular biomarkers are introduced, we believe that using simple but useful biomarkers such as anthropometric evaluations e.g. TSF and AMA or blood visceral proteins as well as dynamic biomarkers should be used routinely to identify protein metabolic impairment at the bedside of chronic patients and the involvement of a specialised professional capable of prescribing tailored nutritional intervention for each patient.

Repeated controls of plasma proteins can also monitor the success or failure of therapeutic procedures. The preservation of protein metabolism by specific therapy has an important clinical impact because it maintains the functions of global metabolism, helping traditional therapy to work properly.

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