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Association between the uremic toxins indoxyl-sulfate and p-cresyl-sulfate with sarcopenia and malnutrition in elderly patients with advanced chronic kidney disease

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ABSTRACT

Background: in patients with chronic kidney disease (CKD) indoxyl sulfate (IS) and p-cresyl sulfate (PCs) may induce sarcopenia either directly or via systemic inflammation. We evaluated whether IS and PCs were associated with: sarcopenia, systemic inflammation and nutritional status. Methods: we examined cross sectionally 93 patients with advanced CKD. Sarcopenia was identified according to EWGSOP2 definition. Malnutrition was assessed by Malnutrition Inflammation Score (MIS) and Protein Energy Wasting syndrome (PEW). Inflammatory status was assessed by dosing: CRP, IL6, TNFα, MCP1, IL10, IL17, IL12p70. Results: we did not find any association of sarcopenia with IS and PCs. IS was associated with LogTNFα and LogMCP-1 in the overall cohort ($r = 0.30$, $p = 0.0043$; $r = 0.22$ $p = 0.047$) and in not sarcopenic patients ($r = 0.32$, $p = 0.0077$; $r = 0.25$, $p = 0.041$). PCs was associated with LogIL10 and LogIL12p70 in sarcopenic patients ($r = 0.58$, $p = 0.0042$; $r = 0.52$, $p = 0.013$). IS was higher in patients without PEW ($p = 0.029$), while PCs was higher in patients with PEW ($p = 0.0040$). IS and PCs were not different in patients with normal or increased MIS. Conclusions: IS and PCs were not associated with sarcopenia, although they were both associated with some inflammatory pathways. Notably, we found a positive association of PCs with PEW syndrome.

1. Introduction

Sarcopenia is characterized by excessive loss of muscle strength and mass. Clinically it is associated with physical disability, poor quality of life and worse overall prognosis (Fielding et al., 2011; Morley et al., 2001). Among subjects affected by chronic kidney disease (CKD) sarcopenia is a prevalent condition and is associated with increased morbidity and mortality (Harada et al., 2017; Androga et al., 2017; Isoyama et al., 2014; Pereira et al., 2015).

Uremic sarcopenia has a multifactorial origin (de Souza et al., 2017), among others, two uremic toxins: indoxyl sulfate and p-cresyl sulfate, that accumulate because of gut dysbiosis and progressive reduction of kidney function, may independently contribute to its development.

Indoxyl sulfate (IS) is a protein-bound uremic toxin that derives from tryptophan metabolism (Barreto et al., 2009). IS has been associated with various complications of CKD such as: cardiovascular and cerebrovascular disease, progression of kidney disfunction, kidney osteodystrophy and increased mortality (Barreto et al., 2009; Stinghen et al., 2014; Hirata et al., 2015; Barreto et al., 2014). Recent research suggests that IS may be involved also in the development of uremic sarcopenia. In an animal model of CKD, Sato and co-workers have shown that IS accumulates in muscle cells and induces metabolic alterations that lead to mitochondrial dysfunction (Sato et al., 2016). In myoblast cells IS increases the expression of reactive oxygen species (ROS) and inflammatory cytokines (TNF-α, IL-6, and TGF-β1) (Enoki et al., 2016a). These factors cause muscular atrophy by inducing the expression of myostatin and atrogin-1 (Enoki et al., 2016a). Moreover, in a recent study, Rodrigues et al. have shown that IS induces apoptosis of myoblasts and skeletal muscle mass reduction in an in vitro model (Rodrigues et al., 2020).

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p-Cresyl Sulfate (PCs) is a toxin derived from tyrosine and phenylalanine metabolism, with kidney excretion (Evenepoel et al., 2009; Nallu et al., 2017). The accumulation of PCs in CKD has been associated with the progression of kidney disease by inducing inflammation, damaging kidney tubular cells (Shimizu et al., 2011; Sun et al., 2013), promoting kidney fibrosis (Watanabe et al., 2013; T M et al., 1997) and stimulating the progression of glomerular sclerosis (Niwa and Ise, 1994). In a murine model Koppe et al. demonstrated that PCs inhibits insulin-stimulated glucose uptake and, by activating ERK kinase, it decreases insulin signalling pathways (Koppe et al., 2013). Moreover, PCs suppresses insulin-induced phosphorylation of Akt, decreasing muscle protein synthesis and inducing protein degradation. Notably, since IS does not seem to influence Akt phosphorylation (Enoki et al., 2016a), the overall effects of IS or PCs on muscle atrophy look like to be independent (Watanabe et al., 2019).

In this paper we explored whether IS and PCs may be associated with sarcopenia in a cohort of older patients affected by advanced CKD. Furthermore, we evaluated whether there was any association between these two toxins with systemic inflammation and nutritional status.

2. Materials and methods

2.1. Patients characteristics

We evaluated in a cross-sectional study 93 prevalent CKD patients from 9/2016 to 3/2018. We asked to participate to the study to all eligible patients that attended the clinic during the enrollment period, when they came for a control visit.

Patients were selected according to the following criteria: age $\geq$ 65 years, 8 $\leq$ eGFR $\leq$45 ml/min/1.73m^2, variations of eGFR $\leq$2 ml/min/1.73/m^2 in 6 months. eGFR was calculated by modified CKD-EPI (Skali et al., 2011; Florkowski and Chew-Harris, 2011). Exclusion criteria were: kidney replacement therapy (dialysis or kidney transplantation); active cancer; cirrhosis and/or ascites; symptomatic heart failure (NYHA class III - IV); nephrotic syndrome; hypo- or hyperthyroidism; malabsorption; inability to cooperate; symptomatic infection in the last two months; current treatment with immunosuppressants and those that were hospitalized in the previous three months.

Physical examination, medical history and nutritional status were assessed at study visit. Twenty-four hours urinary collection was started in the morning of the day preceding the visit. Blood samples were collected the day of the visit, in the morning and after fasting for at least 12 h. The study was conducted according to the ICP Good Clinical Practices Guidelines and to the declaration of Helsinki and it was approved by the Ethics Committee of our Institution (approval document 347/2010, PROVE: PROteinuria and Vascular End points). In order to participate to the study all patients signed an informed consent, as specified in the ICMJE recommendations.

2.2. Assessment of sarcopenia

Sarcopenia was defined in accordance with the criteria of the European Working Group on Sarcopenia in Older People (EWGSOP2) (Cruz-Jentoft et al., 2019).

Muscle strength was evaluated as handgrip strength by using Jamar hand dynamometer (Sammons Preston Inc., Bolingbrook, IL). Handgrip strength was considered reduced for values <16 kg in females and <27 kg in males (Cruz-Jentoft et al., 2019).

Reduced muscle mass was defined as a reduction of mid-arm muscle circumference (MAMC) > 10% respect to the fiftieth percentile of the reference population [26].

Severity of sarcopenia was evaluated by 4 m gait speed test. We adopted a speed >1,25 m/s as a threshold for normality (Guralnik et al., 1995).

2.3. Anthropometry and nutrition

We measured: body weight, height, body mass index (BMI, calculated according to Quetelet Index (kg/m2)), waist circumference (WC), mid-arm circumference (MAC) and calf circumference (CC).

Tricipital and bicipital skinfold (TST; BST) were evaluated with Harpenden caliper. Mid Arm Muscle Circumference (MAMC) was determined on the dominant arm as follows: MAMC (cm) = M AC (cm) $-$ ($\pi \times$ TST (cm)).

Nutritional status was assessed either by the presence of protein-energy wasting syndrome (PEW) or by calculating malnutrition inflammation score (MIS). PEW was assessed according to the criteria defined by the International Society of Renal Nutrition and Metabolism (Yasui et al., 2016). Malnutrition-inflammation score (MIS): is a validated scoring system for the assessment of malnutrition in patients with advanced CKD, patients with MIS > 7 were categorized as malnourished (Kalantar-Zadeh et al., 2001).

Protein intake was estimated by normalized protein catabolic rate (nPCR) that was derived by 24 h urinary urea excretion (Maroni et al., 1985).

2.4. Biochemistry

Biochemical analyses (in serum and urines) for the evaluation of kidney function, metabolic and nutritional status were performed at the central laboratory of our Institution.

2.5. Measurment of uremic toxins

Serum concentration of free IS and PCs have been determined by High Performance Liquid Chromatography (HPLC) and fluorescence detection (FLD) (Pretorius et al., 2013) at the laboratory of biochemistry at the University of Pavia.

2.6. Measurement of serum cytokines

Serum cytokines concentration were measured at the laboratory of nephrology of our Institution. Values were evaluated in duplicate by using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions.

We used these specific kits: Human IL-10 ELISA Kit EHIL10 (Invitrogen, Thermo Fisher Scientific, Monza, Italy), Quantikine ELISA Human CCL2/MCP-1 Immunoassay DCP00, Quantikine ELISA Human IL-12 p70 Immunoassay D1200 (all R&D Systems, Space, Milano, Italy) Human TNF-alpha ELISA Kit (Thermo Fisher Scientific, Monza, Italy). Quantikine ELISA Human IL-17 Immunoassay, Quantikine HS ELISA Human IL-6 Immunoassay HS600B (R&D Systems, Space, Milano, Italy). Zero was included in each resulting curve as the last standard value. Results were validated by using Quantikine Immunoassay Control Group 1–4 or 10 (R&D Systems, Space, Milano, Italy). Absorbance readings were measured at 450 nm by spectrophotometer (Xenius Safas, Monaco).

2.7. Statistical analysis

All data are expressed as mean $\pm$ SD or median $\pm$ IQR as appropriated. Comparisons of normally distributed variables was done using Student's *t*-test while the comparison of not normally distributed ones was done by using Mann-Whitney "U" test. Proportions and categorical variables were compared by using independent chi-squared (χ^2) test or Fisher's exact test. Variables with skewed distribution have been transformed in their base 10 logarithm. Regression analyses were performed by using Pearson or Spearman tests as appropriated. Statistical analysis was carried out with Statview software version 5.0.1.

3. Results

3.1. Characteristics of the population

We reported in Table 1 the characteristics of the overall population as well as the comparison between Src and Not-Src groups. Prevalence of sarcopenia was 25% (23/93). Src were older (84 ± 7 vs 81 ± 9, p = 0.031) and with lower BMI respect to Not-Src (25.1 ± 3.7 vs 29.1 ± 4.5, p = 0.0002). Src and Not-Src did not differ neither for eGFR nor for creatinine clearance. PEW was more prevalent in Src than in Not-Src (43 vs 20%, p = 0.026).

3.2. Biochemical nutritional parameters

In order to evaluate whether there was any difference in nutritional status between Src and Not-Src we compared the two populations for several different nutritional parameters (Table 2).

Src and Not-Src individuals did not differ for any of the biochemical nutritional parameters except for HDL, that was higher in Src patients (58 ± 22 vs 47 ± 19, p = 0.021).

3.3. Uremic toxins and inflammatory markers

We compared plasma concentrations of uremic toxins and/or of inflammatory cytokines between Src and Not-Src patients, but we did not find any significant difference (Table 3). Furthermore, we analyzed whether there was any correlation between IS, PCs and inflammatory cytokines in the overall population as well as in Src and Not-Src groups (Table 4).

In the overall population Log IS was associated with both Log TNFα (r = 0.30; p = 0.0043) and Log MCP-1 (r = 0.22; p = 0.047), while Log PCs was not associated with any of the inflammatory cytokines.

When this analysis was performed in Not-Src and Src separately: Log PCs was positively associated with Log IL17 in Not-Src (r = 0.31; p = 0.013) as well as with Log-IL10 and Log-IL12p70 (r = 0.58; p = 0.0048 and r = 0.52; p = 0.013, respectively) in Src individuals; Log IS was associated with Log TNFα and Log MCP only in Not-Src (r = 0.32; p = 0.0077 and r = 0.25; p = 0.041 respectively).

We evaluated also the correlations between uremic toxins and nutritional status, where we defined malnutrition as the presence of PEW or MIS > 7 (Table 5). PCs and IS had a divergent behavior in their association with PEW. Patients with PEW had higher concentrations of PCs (2.4 ± 2.7 vs 1.6 ± 2.9, p = 0.0040) and lower concentrations of IS (0.5 ± 0.9 vs 0.8 ± 1.2, p = 0.029) respect to those without PEW.

We did not find any difference of IS and PCs with or without documented malnutrition at MIS.

Table 1
Characteristics of the population.

Variables	Overall (n = 93)	Not-Src (n = 70)	Src (n = 23)	P
Age (years)	81 ± 9	81 ± 9	84 ± 7	0.031
BMI	28.1 ± 4.7	29.1 ± 4.5	25.1 ± 3.7	0.0002
Males	66 (71%)	48 (69%)	18 (78%)	0.37
Diabetes	52 (56%)	41 (59%)	11 (48%)	0.37
Previous cardiovascular events	53 (57%)	41 (59%)	12 (52%)	0.54
eGFR (mL/min/1,73m^2)	23 ± 15	23 ± 16	21 ± 11	0.13
Creatinine Clearance (mL/min/1,73m^2)	24 ± 19	24 ± 23	20 ± 20.3	0.12
MIS	5 ± 5	5 ± 4	6 ± 7	0.20
PEW, n (%)	24 (26%)	14 (20%)	10 (43%)	0.026
Variables related to Sarcopenia				
Reduced Handgrip strength, n (%)	60 (64%)	37 (53%)	23 (100%)	<0.0001
Reduced MAMC, n (%)	31 (33%)	8 (11%)	23 (100%)	<0.0001
Reduced Gait Speed Test, n (%)	67 (72%)	46 (66%)	21 (91%)	0.018

BMI, body mass index; eGFR, estimated glomerular filtration rate; MIS, malnutrition inflammation score; PEW, protein energy wasting; MAMC, mid arm muscle circumference.

Table 2
Biochemical and other nutritional parameters.

Variables	Overall cohort (n = 93)	Not-Src (n = 70)	Src (n = 23)	p
Serum parameters				
Albumin (g/dL)	4.0 ± 0.3	4.0 ± 0.3	4.1 ± 0.4	0.47
Prealbumin (mg/dL)	28 ± 5	29 ± 5	27 ± 6	0.36
Total cholesterol (mg/dL)	156 ± 42	156 ± 36	166 ± 51	0.31
HDL (mg/dL)	48 ± 19	47 ± 19	58 ± 22	0.021
LDL (mg/dL)	80 ± 33	80 ± 35	87 ± 28	0.4
Triglycerides (mg/dL)	128 ± 56	133 ± 59	116 ± 44	0.21
Transferrin (mg/dL)	231 ± 41	232 ± 41	226 ± 43	0.56
Vitamin D 25 OH (ng/ml)	27 ± 20	28 ± 17	26 ± 30	0.62
24 h urine collection				
Urinary urea (mg/24 h)	16,570 ± 5433	16,721 ± 5329	16,112 ± 5922	0.65
Urinary creatinine (mg/ 24 h)	899 ± 312	918 ± 318	843 ± 294	0.32
nPCR (mg/kg/24 h)	726 ± 336	749 ± 329	688 ± 293	0.64
Calories intake (kcal/kg)	21.0 ± 8.5	24.9 ± 7.5	20.3 ± 8.6	0.02

HDL, High density lipoprotein; LDL, Low Density Lipoprotein; nPCR, normalized Protein Catabolic Rate.

Table 3
Uremic toxins and inflammatory markers in Not-Src and Src patients.

	Overall cohort (n = 93)	Not-Src (n = 23)	Src (n = 70)	p
Uremic toxins				
PCs (μmol/l)	2.1 ± 3.0	2.3 ± 3.0	1.8 ± 3.4	0.40
IS (μmol/l)	0.7 ± 1.2	0.75 ± 1.5	0.6 ± 0.9	0.20
Pro-inflammatory markers				
CRP (mg/dl)	0.21 ± 0.32	0.23 ± 0.32	0.20 ± 0.29	0.77
IL-6 (pg/ml)	3.6 ± 3.78	3.4 ± 3.7	4.1 ± 4.2	0.62
TNFα (pg/ml)	13.9 ± 9.9	13.6 ± 11.1	14.6 ± 7.2	0.59
MCP-1 (pg/ml)	412.8 ± 243.9	410.9 ± 218.4	428.9 ± 314.6	0.08
IL17 (pg/ml)	0.39 ± 1.15	0.39 ± 1.26	0.37 ± 0.78	0.77
Anti-inflammatory markers				
IL12p70 (pg/ml)	0.85 ± 1.96	0.85 ± 1.9	0.8 ± 2.2	0.08
IL-10 (pg/ml)	1.68 ± 7.0	1.9 ± 6.2	1.2 ± 7.6	0.43

PCs, p-cresyl sulfate; IS, indoxyl sulfate; CRP, C-reactive protein; IL-6, interleukin 6, TNFα, Tumor nercosis factor alpha; MCP-1, Monocyte chemotactic protein-1; IL-10, interleukin 10; IL12p70, interleukin 12p70; IL17; interleukin 17.

4. Discussion

Our first aim was to explore whether levels of IS and PCs were associated with sarcopenia in a cohort of older patients with advanced CKD. In this respect, we did not find any correlation between IS and PCs with sarcopenia. Furthermore, we evaluated whether there was any association between these two uremic toxins with systemic inflammation. In the overall cohort we found a moderate but significant correlation between IS and TNFα and MCP-1. However, when considering patients with and without sarcopenia separately, these correlations were maintained only in Not-Src group. In the overall cohort there were no correlations of PCs with inflammatory cytokines. Nevertheless, PCs was positively correlated with IL12p70 in Src and with IL17 Not-Src.

Table 4
Correlations between uremic toxins and pro and anti-inflammatory markers.

	Overall cohort (n = 93)		Not-Src (n = 70)		Src (n = 23)	
Log PCs	r	p	r	p	r	p
Log CRP (mg/dl)	0.038	0.7	0.10	0.39	0.24	0.27
Log IL-6 (pg/ml)	0.082	0.45	0.11	0.37	0.007	0.97
Log TNFα (pg/ml)	0.036	0.74	0.14	0.25	0.37	0.09
Log MCP-1 (pg/ml)	0.058	0.60	0.18	0.14	0.088	0.71
Log IL-10 (pg/ml)	0.007	0.95	0.11	0.35	**0.58**	**0.0042**
Log IL12p70 (pg/ml)	0.28	0.069	0.225	0.067	**0.52**	**0.013**
Log IL 17 (pg/ml)	0.19	0.08	**0.31**	**0.013**	0.24	0.28
Log IS						
Log CRP (mg/dl)	0.028	0.79	0.046	0.71	0.025	0.91
Log IL-6 (pg/ml)	0.065	0.56	0.019	0.88	0.39	0.087
Log TNFα (pg/ml)	**0.30**	**0.0043**	**0.32**	**0.0077**	0.27	0.21
Log MCP-1 (pg/ml)	**0.22**	**0.047**	**0.25**	**0.041**	0.15	0.52
Log IL-10 (pg/ml)	0.07	0.51	0.04	0.75	0.14	0.54
Log IL12p70 (pg/ml)	0.072	0.50	0.085	0.49	0.029	0.89
Log IL 17 (pg/ml)	0.094	0.38	0.15	0.23	0.101	0.66

PCs, p-cresyl sulfate; IS, indoxyl sulfate; CRP, C-reactive protein; IL, interleukin, TNFα, Tumor nercosis factor alpha; MCP-1, Monocyte chemotactic protein-1; Significant correlations are highlighted in bold.

Table 5
Correlations between uremic toxins and nutritional status.

Variables	PEW (n = 24)	No PEW (n = 69)	P
PCs (μmol/l)	2.4 ± 2.7	1.6 ± 2.9	**0.0040**
IS (μmol/l)	0.5 ± 0.9	0.8 ± 1.2	**0.029**

Variables	MIS > 7 (n = 26)	MIS < 7 (n = 67)	P
PCs (μmol/l)	2.2 ± 3.8	2.1 ± 2.8	0.54
IS (μmol/l)	0.7 ± 0.9	0.7 ± 1.4	0.92

PCs, p-cresyl sulfate; IS, indoxyl sulfate; PEW, Protein Energy Wasting; MIS, Malnutrition Inflammation Score Significant correlations are highlighted in bold.

Finally, we evaluated whether there was any association between IS and PCs and nutritional status. Regarding the association with PEW we found opposite results. Notably IS was higher in patients without PEW, while PCs was higher in patients with PEW.

We did not find any difference in IS and PCs concentration in patients classified as well or malnourished according to MIS.

To our knowledge, this is the first study exploring a possible association between the uremic toxins IS and PCs with sarcopenia in humans affected by advanced CKD but not yet in dialysis. Preliminary studies, conducted on murine or in vitro models, demonstrated that IS and PCs might contribute to the insurgence of sarcopenia in independent ways (Watanabe et al., 2019). IS accumulates in muscle cells and induces metabolic alterations that lead to mitochondrial dysfunction (Sato et al., 2016). In myoblast cells IS increases the expression of ROS and inflammatory cytokines (TNF-α, IL-6, and TGF-β1) causing muscular atrophy (Enoki et al., 2016a). In an in vitro study, Rodrigues et al. showed that IS induces apoptosis of myoblasts and muscle mass reduction (Rodrigues et al., 2020). PCs has been demonstrated to inhibit insulin-stimulated glucose uptake and, by activating ERK kinase, It may inhibit the metabolic pathways mediated by insulin (Koppe et al., 2013). Furthermore, PCs, can inhibit protein synthesis and induce muscular protein degradation by suppressing insulin-induced phosphorylation of Akt (Koppe et al., 2013).

Overall, our study does not seem to confirm these preliminary results. Indeed, we did not find any association among IS, PCs and sarcopenia in humans affected by advanced CKD.

Sato and co-workers (Sato et al., 2016) described a reduction of number and viability of myoblast cells when they were cultured in vitro and incubated with IS. However, this effect was significant only for very high concentrations of IS (0,5–1 mM) in the incubation liquid, values that are by far higher than those that were actually measured in the blood of CKD patients. Therefore, it is plausible that in order to induce intracellular toxicity it is necessary to reach very high extracellular concentrations of IS, even if the kinetic regulating the balance between intra and extracellular IS is still scarcely known. In the same paper Sato and colleagues (Sato et al., 2016) described also an inverse correlation between plasmatic IS and skeletal muscle mass in patients with. Therefore, although intracellular IS concentration may be better correlated to muscular toxicity, in patients already on dialysis plasmatic IS may be considered a reliable surrogate. This may be due to the fact that in dialysis IS concentrations are much higher than at lower stages of CKD, but also by the onset of concomitant conditions, as metabolic acidosis and malnutrition, that have a catabolic effect and may enhance muscular toxicity of IS. The discrepancy between our results and those of previous preclinical studies may also depend on the fact that in animal and in-vitro models the experimental conditions are strictly controlled. Instead, although the characteristics of our population were fairly homogeneous, we could not correct for all confounding factors. In particular, we did not stratify our cohort according to nutritional status that may represent a specific condition as suggested by our results. IS and PCs are oppositely associated with PEW, since IS was higher in No-PEW group while PCs was higher in PEW. Conversely, the concentrations of the two toxins did not differ in patients with or without malnutrition at MIS. Although IS was not associated with e GFR (r = 0,052; p = 0,623) while PCs it was (r = 0,358; p = 0,0005), it seems unlikely that this discrepancy may have influenced their different behaviours since eGFR was not associated with PEW (23.0 ± 15.5 vs 21.5 ± 13.0, p = 0.44). Since we did not specifically address the synthesis and intestinal absorption of the two toxins (gut microbiota composition, dietetic amino acids intake etc.), we cannot exclude that this may have somehow influenced our results. However, we cannot even exclude that overall effects of IS and PCs on energy protein metabolism may be truly independent from each other. Indeed, IS induces mitochondrial dysfunction, increases the production of reactive oxygen species and inflammatory cytokines and provokes muscular atrophy but is not associated with body fat that is a key component for the definition of PEW (Sato et al., 2016; Enoki et al., 2016a; Rodrigues et al., 2020). On the other hand, PCs reduces protein synthesis and induces protein degradation by inhibiting insulin-stimulated signalling pathways (Koppe et al., 2013), pre-conditions that may be strictly correlated with the development of PEW. Thereof, the opposite associations of IS and PCs with PEW could have a pathophysiological explanation that may deserve to be specifically explored by further research.

The pathogenesis of uremic sarcopenia is multifactorial: insulin resistance, malnutrition, alterations of the mitochondrial metabolism, excess lactic acid production and carnitine deficiency (Bailey et al., 2006; Zhou et al., 2018) may all play a role in the onset of sarcopenia. In addition, also abnormalities of vitamin D metabolism, secondary hyperparathyroidism and other mineral and bone disorders related to CKD, have been associated to muscular weakness in uremic patients (Gordon et al., 2012). Also systemic inflammation has been reported to contribute to the loss of muscle mass in CKD patients (Cheung et al., 2010; Raj et al., 2008; Du et al., 2004). During muscle wasting, abnormally high levels of ROS and inflammatory cytokines are produced in skeletal muscle (Sriram et al., 2011; Powers et al., 2005). An increase in TNF-α stimulates myostatin expression through NF-κB pathway, which further induces myostatin expression and release of IL-6 in striated muscle cells (Zhang et al., 2013). We explored for the first time the interactions between IS and PCs with a wide spectrum of pro (IL-6, TNFα, MCP-1 and IL-17) (Kim et al., 2010; Schaap et al., 2009; Ikizler et al., 1999; Ramani and Biswas, 2016) and anti-inflammatory (IL10 and IL12p70) (Stenvinkel et al., 2005; Teng et al., 2015) cytokines in patients affected by advanced CKD either with or without sarcopenia. We evaluated IL-6 and TNF-α because both IS and PCs increase the

expression of these inflammatory cytokines causing muscular atrophy (Enoki et al., 2016b). The expression of MCP-1 in proximal tubular cells is upregulated by IS upregulates through production of reactive oxygen species (ROS) and activation of: NF-κB, p53, ERK, and JNK (Shimizu et al., 2012). CRP and IL-17 account for the effect of inflammation, which is an important part of uremic milieu that can induce muscle catabolism (Pajek et al., 2018; Pecoits-Filho et al., 2002). Conversely IL-10 and IL-12 are regulators of the immune system by limiting the inflammatory response (Teng et al., 2015, Enoki et al., 2016a). On this respect we found an association of PCs with IL12p70 and IL10 in sarcopenic patients as well as with IL10 in those that were not sarcopenic. Furthermore, we found that PCs was associated with TNFα and MCP-1 either in the overall cohort as well as in not sarcopenic patients.

In our study both IS and PCs were associated with PEW but not with malnutrition determined by using MIS. PEW is a multifactorial syndrome that combines protein energy wasting and malnutrition in individuals with chronic kidney disease (Fouque et al., 2008). According to its defining criteria, PEW can be only present or absent without any other grading. Conversely, MIS is based on the evaluation of 10 domains (7 components of the subjective global assessment, body mass index, serum albumin and transferrin) each of whom is graded for three levels of severity. While PEW has a dichotomous definition, MIS is more gradual and presents a progressive increase of severity up to the maximum score of 30. Therefore, it may be that malnutrition defined by PEW intercepted a different, and probably sicker, group of patients respect to those that have been selected by a definition based on a MIS $>$ 7. Whether this was true, we could hypothesize that the variation of uremic toxins that we observed in PEW are associated with a severer degree of malnutrition. Since in CKD patients PEW is more frequent in sarcopenic individuals (Vettoretti et al., 2019) it is possible that uremic toxins may indirectly influence the development of sarcopenia by inducing PEW. In particular, these effects may be mediated by the blockade of the metabolic pathway of insulin. In fact, PCs inhibits insulin-stimulated glucose uptake and, by activating ERK kinase, it decreases insulin signalling pathways (Koppe et al., 2013). Moreover, PCs suppresses insulin-induced phosphorylation of Akt, decreasing muscle protein synthesis and increasing protein degradation. Overall, PEW has a multifactorial pathophysiology. It is therefore possible that, although IS and PCS are associated with PEW and PEW with sarcopenia, the effects of uremic toxins on the development of sarcopenia might be diluted by the other factors determining the onset of PEW.

Finally, the amount of IS and PCs depends on a multiplicity of factors, as individual eGFR or the composition of intestinal microbiota (Evenepoel et al., 2009). Thus, although our patients with and without sarcopenia have a comparable glomerular filtrate it is still possible that other phenomena, which have not been specifically addressed in our study, may have influenced the concentration of uremic toxins in patients with or without sarcopenia.

Our study has some limitations. First, our cohort is made of very elderly subjects and could have interfered with our results because changes due to uremia and disease may overlap. Second, the relatively small sample size may have biased the associations that we observed. Furthermore, we did not evaluate dietetic intake of amino acids as well as the enteric production of uremic toxins that may depend on the specific composition of gut microbiota. However, we applied stringent selection criteria that allowed us to limit main confounding factors. Indeed, we excluded the majority of conditions that may have independently influenced nutritional status and/or inflammation.

In conclusion, in older patients affected by advanced CKD we did not observe any association between IS and PCs with sarcopenia. However, we found some correlations between IS and PCs with inflammatory cytokines that may influence nutritional status and sarcopenia in this group of patients. Notably, we found a positive association of PCs with PEW syndrome, a condition that is strictly correlated with sarcopenia.

Our study represents a seminal phase for future insights. In particular, it will be interesting to explore in-depth the influence of IS and PCs on systemic inflammation and on the development of PEW in patients affected by advanced CKD.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare no conflict of interest.

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