ORIGINAL RESEARCH

Myostatin and Insulin-Like Growth Factor 1 Are Biomarkers of Muscle Strength, Muscle Mass, and Mortality in Patients on Hemodialysis

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Objective: Muscle strength is frequently altered in hemodialysis patients. In the present work, five potential muscle biomarkers have been studied in their ability to assess muscular strength, muscular mass and to predict mortality of hemodialysis patients: activin-A, procollagen III N-terminal peptide, follistatin, myostatin and insulin-like growth factor-1 (IGF-1).

Design and methods: Three independent cohorts of prevalent hemodialysis patients (2 from Liège, Belgium and 1 from Marseille, France) were considered in this observational prospective study. The biomarkers were first measured in the Liege1 cohort. Two of them, myostatin and IGF-1, were then assessed in the whole population of patients (Liege1, Liege2 and Marseille). Muscle strength was assessed with handgrip strength (HGS) and muscle mass with bioimpedance analysis. One-year mortality predictive value of biomarkers was also studied in the Liège1 and Marseille cohorts.

Results: In the Liège1 cohort (n=67), HGS was only associated with concentrations of myostatin and IGF-1. These associations were confirmed in the whole population of 204 patients (r=0.37, $P<0.001$ and r=0.46, $P<0.001$, respectively) and remained significant ($P<0.05$) in multivariable models. The association between muscle mass and concentrations of myostatin and IGF-1were also significant. The ability of myostatin, IGF-1 and serum creatinine to detect a low HGS compared by Receiver Operating Characteristic curves analysis were not significantly different. Both myostatin and IGF-1 had a significant and comparable area under the curve to predict one-year mortality: 0.73 (95% CI: 0.64 to 0.83) and 0.72 (95% CI: 0.61 to 0.82), respectively.

Conclusion: Our results suggest that myostatin and IGF-1 are two biomarkers of interest to assess muscle status of dialysis patients. Both biomarkers are associated with HGS, muscular mass, and one-year mortality.

Introduction

MUSCLE MASS AND muscle strength are frequently altered in patients on hemodialysis,[1,2] a condition associated with poor outcomes.[3,4] Detecting sarcopenia is crucial as it can be reversed using nutritional approaches and physical exercising.[5,6] Measuring muscle strength or muscle mass requires specific tools and is time-consuming. There is no consensus regarding assessment of muscle function in hemodialysis clinical routine.[5] In a quest to facilitate sarcopenia screening and follow-up, biomarkers are of major interest.

Until now, the best biomarker to assess muscle function in patients on hemodialysis is serum creatinine (SCr), but with important limitations as creatinine is influenced by residual renal function and dialysis modality.[7] New biomarkers related to muscle function have emerged in general population.[8] Insulin-like growth factor 1 (IGF-1) induces protein synthesis and muscle production by activating the akt/mTOR pathway.[9,10] Conversely, both

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activin A and myostatin are blocking the akt/mTOR pathway.[11-13] Follistatin is an extracellular glycoprotein that neutralizes the activity of myostatin.[14] Procollagen III N-terminal peptide (PIIINP) is the part of procollagen III released in the circulation when collagen III is synthetized in the muscle.[15] In the present work, these molecules were measured in serum of hemodialysis patients and compared with functional (handgrip strength [HGS]) and structural tests (muscle mass by bioimpedance analysis [BIA]). We also tested their ability to predict mortality within 1-year.

Methods

Population

In this observational prospective study, three independent cohorts of prevalent hemodialysis patients were explored. Two cohorts were from Liège, Belgium (referred as Liège1 [n = 67] and Liège2 [n = 56]). The third cohort was from Marseille, France (referred as Marseille [n = 81]). In Liège, the protocol was approved by the Ethics Committee of our institution "Comité d'éthique hospitalo-facultaire universitaire de Liège" (number B707201525774). In Marseille, this study obtained approval from the Health Research Data Processing Advisory Committee and the French National Commission for Data Protection and Liberties. The study was in accordance with the ethical standards of Helsinki declaration, and signed written consent was obtained for each patient.

Measurement of Biomarkers

Samples were drawn from participating patients before the dialysis session. Samples were allowed to clot 30 minutes and then centrifuged at 45,000 RPM for 10 minutes. Sera were aliquoted and kept frozen at −80°C until determination. IGF-1 was determined on the IDS-iSYS analyzer (IDS, Boldon, UK). In this chemiluminescent assay, the patient samples are incubated with an acidic solution to dissociate IGF-1 from the binding proteins, and two monoclonal antibodies are used to measure IGF-1. In our hands, the coefficient of variation (CV) was <7%. PIIINP was measured using the radioimmunoassay from Orion Diagnostica (Espoo, Finland), and follistatin, myostatin and activin A were measured using an ELISA from R&D (R&D Systems, Minneapolis, MN). We have extensively analytically validated PIIINP, myostatin, and activin A.[8] The interassay CV ranged from 5.5% to 8.4% for activin A, from 7.2% to 8.9% for myostatin, from 5.2% to 10.1% for PIIINP, and from 7.1 to 9.2% for follistatin. According to R&D, the follistatin assay measures total follistatin concentration (follistatin 288, follistatin 300, and follistatin 315), and the myostatin assay measures the total form of the peptide (only the C-terminal form being bioactive). IGF-1, PIIINP, follistatin, myostatin, and activin A were measured in the same laboratory (Department of Clinical Chemistry, University of Liège) accredited for the ISO 15189 Guideline. Other biological parameters (serum calcium, phosphorus, parathyroid hormone (PTH), C-reactive protein (CRP), albumin, and SCr) were measured locally. Albumin was measured using Bromocresol green in all centers. Because PTH was measured by different assays in the three centers, we considered relative results expressed in multiples of the upper normal limit values, as established by our team.[16]

Handgrip Strength Measurement

Muscle strength was assessed by HGS. HGS was measured in dominant arms on a dialysis day but before connection to the dialysis machine.[17] In the two Liège cohorts, measurements were performed in triplicates, with an interval of 5 seconds between measurements, and the higher value was kept for further analysis.[18] The same Jamar dynamometer (Jamar® Hydraulic Hand Dynamometer, Patterson Medical, Warrenville, IL) with a precision of 0.5 kg was used for all measurements in Liège1 and Liège2. In Marseille, HGS was measured using a quantitative handgrip dynamometer (Kern, Germany). Measurement was performed in the dominant arm, and the value used was the mean of three consecutive measurements. An HGS value below 30 kg in men and 20 kg in women was considered as abnormally low (European Working Group on Sarcopenia in Older People recommendations).[19]

Step by Step Process

In the Liège1 cohort (exploratory cohort), which was used for the first proof-of-concept analyses, associations between the five biomarker candidates and muscle strength were studied. In the second step of analyses, only the biomarkers of interest detected in the Liège1 were tested in the two other cohorts (Liège2 and Marseille, validation cohorts).

Biomarkers and Muscle Mass

The two biomarkers of interest identified in the proof-of-concept cohort were also studied as muscle mass index (MMI) biomarkers in the Marseille cohort. Muscular mass measurements by BIA were available only in this cohort. BIA was performed during the midweek hemodialysis session in the recommended standardized conditions using Z-Hydra® (Bioparhom, France), which is a multifrequency BIA device that allows measurement during a hemodialysis session. The MMI was calculated as muscle mass in kilograms divided by squared height in meters. Patients with limb amputation or pacemaker were excluded (n = 10).

The Effect of Dialysis on Myostatin Concentration

To assess the intradialytic variation of myostatin, we measured and compared its concentration before and after

dialysis in 10 single dialysis sessions performed in 10 Liège1 patients. Among them, 5 patients were dialyzed by hemodialysis and 5 by hemodiafiltration. Results after dialysis were corrected for total extracellular volume change.

Survival Analysis

Finally, the mortality predictive value of the biomarkers was studied in the Liège1 and Marseille cohorts for which 1-year survival data were prospectively followed up.

Statistics

Data are expressed as mean ± standard deviation when distribution was normal and as median with interquartile range [quartile1 to quartile 3] when not. Normality was assessed by the Shapiro-Wilk test. A comparison between cohorts was realized by calculating p-values with analysis of variance for continuous variables, followed by Tukey's Studentized range test to control type 1 error rate. For categorical variables, Pearson's exact chi-square test was used. The correlation between assays and HGS was evaluated by Pearson correlation. Backward and forward multiple regression analysis using $\alpha = 0.05$ as the entry criterion was used to study the potential linear relationship between biomarkers and clinical or biological data. For the comparison of concentration results between assays before and after dialysis, Wilcoxon rank sum test was used to test the equality of the mean relative change between hemodialysis and hemodiafiltration sessions. Univariate survival curves (Kaplan-Meier) were calculated for strata defined by the biomarker cutoff corresponding with the optimal Youden index, based on the receiver operating characteristic (ROC) analysis (with subgroups based on death). Cox proportional hazard regression models were used to study the risk of 1-year mortality associated with biomarkers adjusted for age and gender. ROC curves (with subgroups based once on death and once on decreased HGS) were evaluated for biomarkers of interest. For these analyses, we also compared the combination of myostatin and IGF-1 (logistic regression model) with SCr.

Results

Demographics and biochemistry data for the total studied population (n = 204) and each cohort separately are summarized in Table 1. Briefly, the median age of the total studied population was 71 [58-81] years, 60% of the population were men, and 42% of patient had diabetes. The median HGS was 20 [14-29] kg. A total of 67% of patients had a low muscle strength, as defined previously.[19] Median myostatin and IGF-1 serum concentrations were 2573 [1663-3703] pg/mL and 118 [84-172] μg/L, respectively.

Linear Correlation Between the 5 Biomarkers and HGS in the Exploratory Cohort (n = 67)

A significant association was found between HGS and myostatin and IGF-1 concentrations, but no association was found between HGS and activin A, follistatin, or PIIINP (Table 2). Therefore, myostatin and IGF-1 were further studied (Table 3).

HGS and Concentrations of Myostatin and IGF-1 in the Whole Cohort (n = 204)

The significant associations between HGS and myostatin or IGF-1 were confirmed in the whole population (r = 0.37 [95% confidence interval (CI): 0.25 to 0.48], $P < .0001$ and r = 0.46 [95% CI: 0.35-0.57], $P < .0001$, respectively) (Fig. 1A and 1B, respectively). In the total cohort, we also found a significant correlation between HGS and SCr (r = 0.50 [95% CI: 0.39-0.59], $P < .0001$) (Fig. 1C). To note, correlations between myostatin, IGF-1, SCr, and HGS were not significant in Liège2 (Table 3).

We found a significant linear association (− if negative and + if positive) in univariate analysis ($P < .05$) between myostatin and age (−), gender (higher concentrations in men), height (+), hemodiafiltration (higher concentration compared to hemodialysis), bicarbonates (−), prealbumin (+), center, CRP (−), and HGS (+). In the multivariable model ($R^2 = 0.31$), with myostatin as the dependent variable, a significant association ($P < .05$) was found between myostatin and age, center, CRP, and HGS.

Regarding IGF-1, we found a significant linear association in univariate analysis ($P < .05$) with age (−), height (+), dry weight (+), dialysis vintage (−), hemodiafiltration (higher concentration compared to hemodialysis), bicarbonates (−), phosphates (+), albumin (+), prealbumin (+), and HGS (+). In the multivariable model ($R^2 = 0.41$), a significant association ($P < .05$) was still found between IGF-1 and age, prealbumin, and HGS.

For SCr, we found a significant linear association in univariate ($P < .05$) with age (−), gender (higher concentration in men), height (+), dry weight (+), HGS (+), phosphates (+), albumin (+), bicarbonates (−), prealbumin (+), PTH (+), CRP (−), center, dialysis vintage (+), hemodiafiltration (higher concentration with hemodiafiltration), and residual renal function (−). In the multivariable model ($R^2 = 0.42$), a significant association ($P < .05$) was still found between SCr and HGS, phosphates, PTH, prealbumin, dialysis vintage and residual renal function.

The ability of myostatin, IGF-1, and SCr to detect a decreased HGS was then compared by ROC curve analysis. Both myostatin and IGF-1 had a significant and comparable area under the curve (AUC): AUC = 0.72 (95% CI: 0.65-0.79) for myostatin and AUC = 0.71 (95% CI: 0.63-0.79) for IGF-1. AUC for creatinine was lower although the difference was not significant compared with myostatin and IGF-1: AUC = 0.67 (95% CI: 0.59-0.74) (Fig. 2). Combining IGF-1 with myostatin increased the AUC to 0.76 (95% CI: 0.69-0.83; $P = .0238$, in comparison to AUC of SCr alone), whereas adding SCr to myostatin or IGF-1 did not reach higher AUCs.

Table 1. Demographics and Biochemistry Description of Cohorts

Variables	Whole (n = 204)	Liège1 (n = 67)	Liège2 (n = 56)	Marseille (n = 81)	*P* Value
Age	71 [58; 81]	65 [46; 77]	70 [58; 77]	77 [69; 85]*	<.0001
Gender (% of men)	60	64	55	60	NS
Residual renal function (>200 mL/day) (%)	69	49	88†	72*	<.0001
Dialysis vintage (months)	29.5 [15.0; 54.5]	24 [14.0; 51.5]	33.5 [18.0; 56.0]	32.0 [13.8; 70.0]	NS
Height (cm)	166 ± 10	168 ± 11	166 ± 10	165 ± 9	NS
Dry weight (kg)	71 ± 16	71 ± 17	73 ± 17	69 ± 15	NS
Dialysis time per session (hour)	4.0 ± 0.4	3.9 ± 0.3	3.8 ± 0.4	4.2 ± 0.5*	<.0001
Hemodiafiltration (%)	50	69	100†	0*	<.0001
Diabetes (%)	42	42	41	42	NS
Bicarbonates (mmol/L)	23.5 ± 3.8	21.3 ± 2.8	22.7 ± 2.8†	26.0 ± 3.7*	<.0001
Hemoglobin (g/dL)	10.9 ± 1.2	10.7 ± 1.2	11.4 ± 1.1*	10.7 ± 1.3	.0038
Calcium (mmol/L)	2.20 ± 0.18	2.22 ± 0.18	2.18 ± 0.19	2.19 ± 0.18	NS
Phosphorus (mmol/L)	1.56 [1.25; 1.92]	1.75 [1.35; 2.06]	1.46 [1.19; 1.92]	1.50 [1.2; 1.7]†	.0169
PTH (multiples of the upper normal limit values)	4.9 [2.6; 8.4]	4.8 [2.6; 7.8]	7.8 [5.6; 11.2]*	3.2 [1.8; 5.8]	<.0001
25OH-vitamin D (ng/mL)	30 [21; 39]	38 [27; 45]	21 [13; 31]*	31 [23; 38]	<.0001
CRP (mg/L)	6 [2; 15]	7 [2; 15]	5 [2; 10]	8 [3; 23]	.0489
Creatinine (mg/dL)	7.51 ± 2.61	8.02 ± 2.82	7.74 ± 2.56	6.92 ± 2.37‡	.0283
Albumin (g/L)	38 [35; 40]	40 [38; 42]*	36 [34; 39]	38 [34; 39]	<.0001
Prealbumin (g/L)	0.28 [0.22; 0.33]	0.30 [0.24; 0.35]	0.28 [0.25; 0.32]	0.26 [0.20; 0.33]†	.0066
Handgrip strength	20 [14; 29]	26 [20; 36]*	21 [16; 29]	17 [12; 24]	<.0001
Handgrip strength (men)	25 [19; 34]	32 [22; 40]	26 [20; 31]	20 [14; 26]	<.0001
Handgrip strength (women)	16 [11; 20]	20 [18; 24]	16 [10; 20]	12 [9; 14]	<.0001
Decreased handgrip strength (%)	67	42	66†	88*	<.0001
Myostatin (pg/mL)	2573 [1662; 3703]	3008 [1978; 4295]	3357 [2184; 4892]	1755* [1163; 2670]	<.0001
IGF-1 (μg/L)	118 [84; 172]	152 [88; 208]	123 [90; 168]	104 [76; 152]†	.002

CRP, C-reactive protein; IGF-1, insulin-like growth factor 1; NS, not significant; PTH, parathyroid hormone.
*If $P < .05$ in comparison with the two other cohorts.
†If $P < .05$ in comparison with Liège1.
‡If $P < .05$ in comparison with Liège2.

Linear Correlation Between Myostatin and IGF-1 and Muscle Mass Index

In the 71 patients with BIA measurements, mean MMI was 8.37 ± 1.73 kg/m^2. Serum myostatin and IGF-1 were statistically correlated with MMI: r = 0.30 (95% CI: 0.07-0.50; $P = .0103$) and r = 0.44 (95% CI: 0.23-0.61; $P < .0001$), respectively.

Concentration of Myostatin Before and After the Dialysis Session

In the 10 tested patients, serum myostatin significantly decreased after the dialysis session (median decrease of −12% [−18; −4]). Median concentrations after dialysis were significantly lower than those before the session: from 1007 to 874 pg/mL ($P = .006$). The decrease of myostatin was equivalent between patients treated with hemodialysis or hemodiafiltration.

Table 2. Pearson Correlations Between Biomarkers and Handgrip Strength in the Exploratory Liège1 Cohort (n = 67)

Biomarkers	Correlation (r) (95% CI)	*P* Values
Activin A	−0.09 (−0.33 to 0.16)	NS
Follistatin	0.007 (−0.23 to 0.25)	NS
PIIINP	0.0008 (−0.23 to 0.25)	NS
IGF-1	0.44 (0.22 to 0.61)	.0002
Myostatin	0.50 (0.30 to 0.66)	<.0001

CI, confidence interval; IGF-1, insulin-like growth factor 1; NS, not significant; PIIINP, procollagen III N-terminal peptide.

Concentrations of Myostatin and IGF-1 and One-Year Mortality

One-year survival status was available for 143 patients, from Liège1 and Marseille cohorts (31 nonsurvivors, 112 survivors, 5 lost to follow-up). Characteristics of survivors and nonsurvivors are shown in Table 4. In univariate analysis, a significant difference between survivors and nonsurvivors was found for age, percentage of patients treated by hemodiafiltration, albumin, CRP, prealbumin, HGS, SCr, myostatin, and IGF-1. The ability of biomarkers to predict mortality was assessed by ROC curve analyses. Both myostatin and IGF-1 had a significant and comparable AUC: 0.73 (95% CI: 0.64-0.83) and 0.72 (95% CI: 0.61-0.82), respectively. The more predictive cutoff values (Youden index) were concentrations of 1647 pg/mL for myostatin and of 106.5 μg/L for IGF-1. Including age to myostatin or IGF-1 did not change the AUC. AUC for creatinine was lower, but the difference was not significant: 0.66 (95% CI: 0.58-0.74) (Fig. 3). However, in a model combining both myostatin and IGF-1, the AUC of the ROC curve was significantly better than the AUC of the

Table 3. Pearson Correlations Between Myostatin/IGF-1/Serum Creatinine and Handgrip Strength in the Whole and Different Cohorts

Biomarkers	Whole (n = 204)	Liège1 (n = 67)	Liège2 (n = 56)	Marseille (n = 81)
Serum creatinine	0.50 (0.39 to 0.59) $P < .0001$	0.57 (0.38 to 0.71) $P = .0002$	0.15 (−0.11 to 0.40) NS	0.59 (0.43 to 0.72) $P < .0001$
Myostatin	0.37 (0.25 to 0.48) $P < .0001$	0.50 (0.30 to 0.66) $P < .0001$	0.06 (−0.20 to 0.32) NS	0.54 (0.37 to 0.68) $P < .0001$
IGF-1	0.46 (0.35 to 0.57) $P < .0001$	0.44 (0.22 to 0.61) $P < .0001$	0.18 (−0.09 to 0.42) NS	0.52 (0.34 to 0.66) $P < .0001$

CI, confidence interval; IGF-1, insulin-like growth factor 1; NS, not significant.
Results are provided as correlation coefficient (95% CI) and *P*-value (univariate analysis).

ROC of SCr alone (AUC: 0.76 [95% CI: 0.65-0.86], $P = .0193$) (Fig. S1).

Cox proportional hazards models are presented in Table 5. Myostatin and IGF-1 were associated with mortality in all models, except when prealbumin was considered.

Survival curves were calculated with the cutoff value of biomarkers obtained from the ROC curve analysis (1647 pg/mL for myostatin, 106.5 μg/L for IGF-1, and 7.2 mg/dL for SCr). These curves were significantly different ($P < .0001$) between patients with myostatin concentrations above and below the cutoff value (Fig. 4A). The same result was obtained when comparing the subgroups obtained with IGF-1 concentrations cutoff ($P = .0006$) (Fig. 4B) and comparing the subgroups for SCr ($P = .0034$) (Fig. 4C). After adjusting for age and gender, the survival curves (above and below the cutoffs) remained significantly different from each other for the three biomarkers. Including albumin, CRP, and the percentage of patients treated by hemodiafiltration did not modify the results. Adding prealbumin in the adjusted model, SCr, and IGF-1 was no longer significant (only prealbumin, $P < .0001$), but the difference was still significant for myostatin ($P = .0197$) and prealbumin ($P < .0001$).

Discussion

We report the interest of myostatin and IGF-1 to assess the muscular health of patients on hemodialysis. These two biomarkers were correlated with muscle function and muscular mass and perform similar to creatinine in detecting low HGS. They were also independently associated with a higher risk of 1-year mortality, even after adjustment for gender, age, and other variables such as CRP, albumin, and dialysis modality. Only the adjustment made for prealbumin resulted in myostatin and IGF-1 being not predictive of mortality.

Nowadays, sarcopenia is mostly diagnosed by interviews, scoring, or HGS measurement, but such measurements have limitations, and eventually a single, unique definition

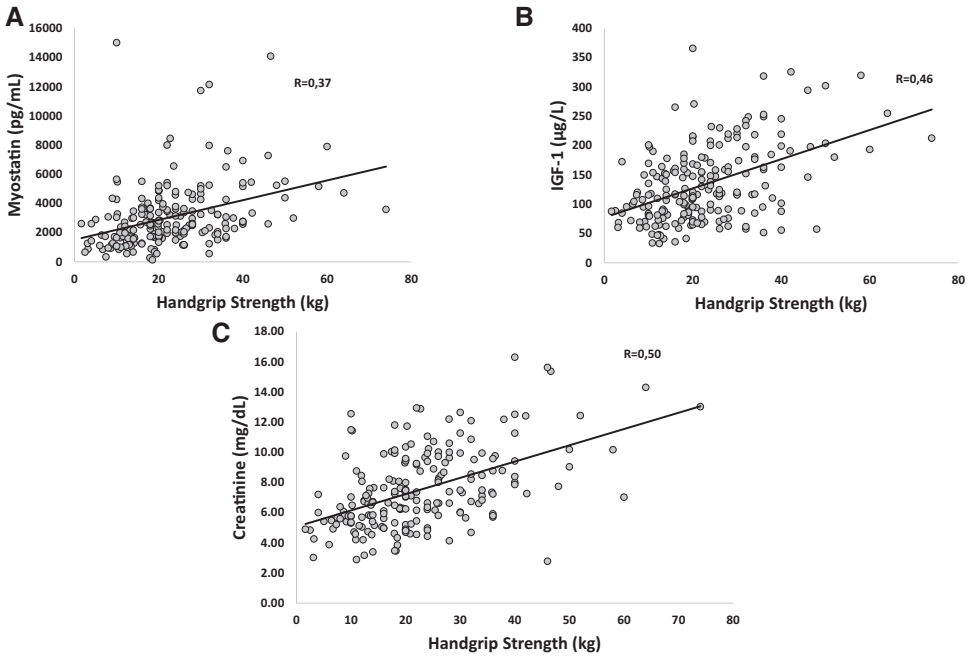

Figure 1. Coefficient of correlation between handgrip strength and myostatin (A), IGF-1 (B), and serum creatinine (C). IGF-1, insulin-like growth factor 1.

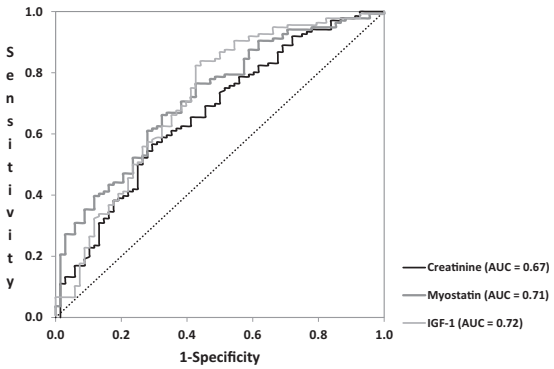

Figure 2. ROC curves for myostatin, IGF-1, and serum creatinine to detect a decreased HGS. AUC, area under the curve; HGS, handgrip strength; IGF-1, insulin-like growth factor 1; ROC, receiver operating characteristic.

of sarcopenia is still lacking in the CKD context.[5] Potential advantages of biomarkers are simplicity and reproducibility.[8]

In our preliminary analysis, we did not confirm the significant (negative) association between HGS and follistatin described by others[14] but found a positive association between HGS and myostatin and IGF-1, which persisted in the multivariable analyses. IGF-1 is a well-know protein that strongly activates protein formation by activating the akt/mTOR pathway in the muscle, leading to an increase in muscle functioning, growth, and repair. Based on these physiological data, a positive association was expected to be found between HGS or muscle mass and IGF-1, and we confirmed here the prior data published regarding patients on hemodialysis.[20-24] Of interest, authors have described an impaired signaling of IGF-1 in a model of CKD mouse,[25] and others evoked a possible resistance to IGF-1 in patients on dialysis.[26]

Myostatin, also named growth and differentiation factor 8, is a member of the superfamily of transforming growth factor β. Myostatin is a chalone protein, i.e., a protein secreted by the muscle itself. Its role is to inhibit the akt/mTOR pathway expressed in myogenic stem cells and proliferating myoblasts by activating specific activin IIb receptors and acts as a robust negative regulator of muscle mass.[27,28] A mutation in myostatin leading to a phenotype of higher muscle mass has been described in a human newborn.[29] The positive association between myostatin and HGS observed in our patients must be discussed. The positive association observed between myostatin and HGS in different populations is still debated in the current literature, with some authors showing negative or no associations[30-38] and others showing a positive one.[11,39-43] Such discrepancies in the literature could be explained, at least in part, by the difference in assays used to measure myostatin. Indeed, myostatin seems particularly difficult to measure (lack of specificity with other proteins from the transforming growth factor β superfamily, lack of sensitivity, and measurement of the full-myostatin protein, whereas only the C terminal fragment is bioactive).[13,40] In this context, it is important to underline that two studies

Table 4. Demographics and Biochemistry Comparison of Patients According to the One-Year Survival Status

Variables	Alive (n = 112)	Death (n = 31)	*P* Value
Age	70 [58; 81]	78 [72; 84]	.006
Gender (% of men)	59	71	NS
Residual renal function (>200 mL/day) (%)	59	68	NS
Dialysis vintage (month)	27 [14.0; 57.0]	38 [24.3; 49.5]	NS
Height (cm)	167 ± 10	164 ± 9	NS
Dry weight (kg)	71 ± 16	67 ± 14	NS
Dialysis time per session (hour)	4.0 ± 0.4	4.1 ± 0.5	NS
Hemodiafiltration (%)	37	10	.0038
Center effect	84% Liège 73% in Marseille	16% Liège 27% in Marseille	NS
Diabetes (%)	40	48	NS
Bicarbonates (mmol/L)	23.8 ± 4.1	24.4 ± 3.9	NS
Hemoglobin (g/dL)	10.7 ± 1.2	10.8 ± 1.3	NS
Calcium (mmol/L)	2.21 ± 0.18	2.20 ± 0.20	NS
Phosphorus (mmol/L)	1.61 [1.29; 1.92]	1.49 [1.23; 1.79]	NS
PTH (multiples of the upper normal limit values)	4.3 [2.1; 6.8]	3.9 [1.1; 6]	NS
25OH-vitamin D (ng/mL)	34 [26; 42]	30 [21; 40]	NS
CRP (mg/L)	5 [2; 14]	12 [3; 35]	.0095
Creatinine (mg/dL)	7.72 ± 2.71	6.24 ± 1.85	.0007
Albumin (g/L)	39 [36; 42]	37 [33; 39]	.0038
Prealbumin (g/L)	0.30 [0.24; 0.36]	0.22 [0.14; 0.27]	<.0001
Handgrip strength	20 [14; 30]	18 [11; 26]	.0478
Myostatin (pg/mL)	2557 [1726; 3537]	1603 [944; 2518]	.0002
IGF-1 (μg/L)	134 [88; 183]	88 [62; 116]	.0003

CRP, C-reactive protein; IGF-1, insulin-like growth factor 1; NS, not significant; PTH, parathyroid hormone.

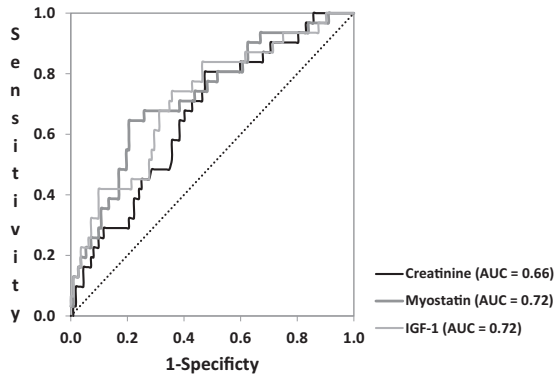

Figure 3. ROC curves for myostatin, IGF-1, and serum creatinine to predict mortality at 1 year (unadjusted). AUC, area under the curve; IGF-1, insulin-like growth factor 1; ROC, receiver operating characteristic.

using mass spectrometry to measure myostatin found a positive association between myostatin and muscle function in nondialysis patients.[39,40] Until now, only scarce and discrepant data are available regarding patients on dialysis. In 2011, Han et al.[21] measured myostatin and IGF-1 in 60 patients undergoing dialysis. They found a negative association between HGS and myostatin using a home-made assay, with poor performance (intraassay CV of 15% and interassay CV of 20%). In 2016, Yamada et al.[41] studied myostatin in 69 Japanese patients treated by peritoneal dialysis, using the same assay as in our study. The authors showed a positive correlation between myostatin and SCr, creatinine excretion, or a model of creatinine kinetics (all indirect measurements of muscle mass).

Only hypotheses can be advanced to explain such a positive correlation. In specific populations, a negative feedback is not necessary because the muscle is already negatively influenced by other pathologic factors: malnutrition, uremic toxins, high oxidative stress, physical inactivity, hypogonadism, and inflammation.[44-47] In other words, the role of "brake" of muscle synthesis by myostatin is unnecessary in these patients who already have a decreased muscle formation for other reasons. Another hypothesis is that myostatin, being produced by the muscle, concentration could be dependent on the number of mature muscle fibers.[34,41] To note, myostatin acts essentially in a paracrine manner, and intramuscular concentrations of myostatin might not be correlated to serum concentrations. Nevertheless, intramuscular dosing of myostatin is impossible in clinical routine. It is also important to consider that myostatin secretion is influenced by numerous factors: its secretion is enhanced by nutritional intakes,[48] inflammation,[49] oxidative stress, or uremic toxins.[46]

Our data confirm previous studies showing that IGF-1 was predictive of mortality in patients on dialysis.[9] For the first time, we showed that myostatin was also associated with mortality. Moreover, myostatin was the only variable

Table 5. Association Between Plasmatic Concentrations of Myostatin and IGF-1 and One-Year Survival: Cox Proportional Hazards Models (n = 143)

	Univariate Analysis	
Included variables	HR [95% CI]	*P* Value
Univariate model		
Age	1.041 [1.012-1.071]*	.0052
Gender	—	NS
Albumin	1.09 [1.02-1.16]*	.0069
Prealbumin†	2.72 [1.84-4.03]*	<.0001
CRP	1.006 [1.002-1.011]*	.0033
Hemodiafiltration	4.61 [1.40-15.2]*	.0119
Serum creatinine*	1.27 [1.07-1.52]	.0073
Myostatin‡	1.81 [1.26-2.58]*	.012
IGF-1§	1.15 [1.06-1.25]*	.0008
Multivariable models		
Model 1		
Age	-	NS
Gender	-	NS
Myostatin	1.49 [1.03-2.15]	.0356
IGF-1	1.11 [1.01-1.21]	.025
Model 2		
Age	-	NS
Gender	-	NS
Albumin	-	NS
CRP	-	NS
Hemodiafiltration	-	NS
Serum creatinine	-	NS
Myostatin	1.49 [1.03-2.15]	.0356
IGF-1	1.11 [1.01-1.21]	.0250
Model 3		
Age	-	NS
Gender	-	NS
Albumin	-	NS
Prealbumin†	2.72 [1.84-4.03]	<.0001
CRP	-	NS
Hemodiafiltration	-	NS
Serum creatinine	-	NS
Myostatin	-	NS
IGF-1	-	NS

CI, confidence interval; CRP, C-reactive protein; HR, Hazard Ratio; IGF-1, insulin-like growth factor 1; NS, not significant.
*HR for a serum creatinine decrease of 1 mg/dL.
†HR for a prealbumin decrease of 0.1 g/L.
‡HR for a myostatin decrease of 1000 pg/mL.
§HR for a IGF-1 decrease of 10 μg/L.

associated with 1-year mortality in a model in which SCr, IGF-1, age, gender, CRP, and albumin were included. Prealbumin seems, as an integrative nutritional biomarker, better than myostatin, IGF-1, and SCr (when continuous variables are considered) or in combination with myostatin (when dichotomized variables are considered) to predict mortality. These results probably reflect the strong and expected association between nutrition and muscle function.

This study must be considered in the light of its limitations. First, our sample is relatively limited, although observations were repeated in three independent centers. Moreover, biomarkers have been measured once, and the interest of repeated measurement should be assessed in further studies. Second, it could be argued that the new

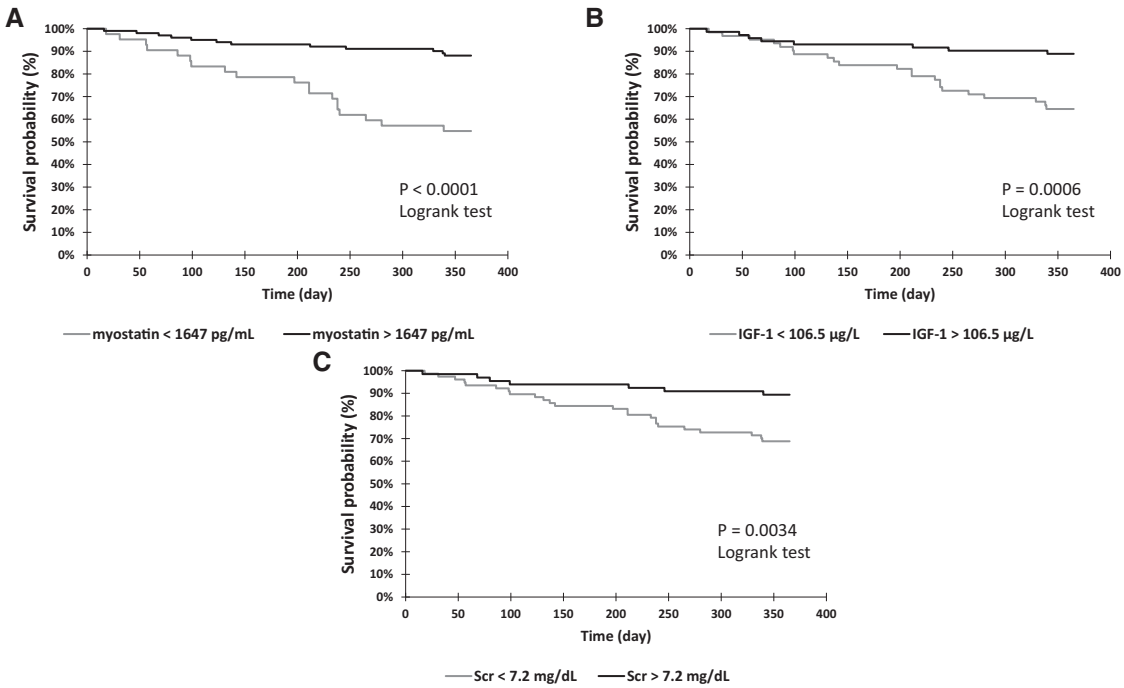

Figure 4. Survival curves within 1 year for myostatin (A), IGF-1 (B), and serum creatinine (C) (unadjusted). IGF-1, insulin-like growth factor 1.

biomarkers do not perform better than SCr. Indeed, the biomarkers have an added value compared with creatinine only when they are considered together. These biomarkers are however less influenced than SCr by other variables, the most important being the absence of association with renal residual function.[50] Also, even if we showed a slight decline of myostatin after the dialysis session (−12%, an expected result according to its molecular weight: 26,000 Da[12]), this decline is much less important than the decline observed with SCr. IGF-1 (molecular weight: 7,600 Da) is not dialyzable because it circulates in the plasma bound to specific protein[51]. Third, because of legal reasons in France, ethnicity could not be considered in the current analysis. Fourth, myostatin and IGF-1 could theoretically be influenced by therapies, notably steroids,[52,53] but treatments were not analyzed in our cohorts. Finally, the assay used to measure myostatin is an ELISA with limitations, and the R&D assay measures the full intact protein. As only the C terminal fragment is bioactive, the real degree of biological activity of myostatin is probably imperfectly reflected by the assay we used. Having said that, the kit we used has been extensively validated from an analytical perspective.[8]

Our results suggest that myostatin and IGF-1 are two biomarkers of interest to assess the muscle status of patients on dialysis. Both biomarkers are associated with HGS, muscular mass, and 1-year mortality. These results should be confirmed in larger cohorts and externally validated.

Practical Application

Myostatin and IGF-1 are two biomarkers of muscle strength and muscle mass in patients on hemodialysis. These biomarkers are also able to detect low HGS values and are also associated with 1-year mortality. These biomarkers could be of clinical use as screening tools of sarcopenia in patients on hemodialysis.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1053/j.jrn.2018.11.010.

References

1. Bataille S, Serveaux M, Carreno E, Pedinielli N, Darmon P, Robert A. The diagnosis of sarcopenia is mainly driven by muscle mass in hemodialysis patients. *Clin Nutr.* 2017;36:1654-1660.
2. Kopple JD, Kim JC, Shapiro BB, et al. Factors affecting daily physical activity and physical performance in maintenance dialysis patients. *J Ren Nutr.* 2015;25:217-222.
3. Kittiskulnam P, Chertow GM, Carrero JJ, Delgado C, Kaysen GA, Johansen KL. Sarcopenia and its individual criteria are associated, in part, with mortality among patients on hemodialysis. *Kidney Int.* 2017;92:238-247.
4. Vogt BP, Borges MC, Goés CR, Caramori JC. Handgrip strength is an independent predictor of all-cause mortality in maintenance dialysis patients. *Clin Nutr.* 2016;35:1429-1433.
5. Carrero JJ, Johansen KL, Lindholm B, Stenvinkel P, Cuppari L, Avesani CM. Screening for muscle wasting and dysfunction in patients with chronic kidney disease. *Kidney Int.* 2016;90:53-66.
6. Manfredini F, Mallamaci F, D'Arrigo G, et al. Exercise in patients on dialysis: a Multicenter, Randomized clinical trial. *J Am Soc Nephrol.* 2017;28:1259-1268.
7. Moreau-Gaudry X, Guebre-Egziabher F, Jean G, et al. Serum creatinine improves body mass index survival prediction in hemodialysis patients: a 1-year prospective cohort analysis from the ARNOS study. *J Ren Nutr.* 2011;21:369-375.
8. Cavalier E, Beaudart C, Buckinx F, Bruyère O, Reginster JY. Critical analytical evaluation of promising markers for sarcopenia. *Eur Geriatr Med.* 2016;7:239-242.
9. Jia T, Axelsson TG, Heimbürger O, et al. IGF-1 and survival in ESRD. *Clin J Am Soc Nephrol.* 2014;9:120-127.
10. Sun DF, Chen Y, Rabkin R. Work-induced changes in skeletal muscle IGF-1 and myostatin gene expression in uremia. *Kidney Int.* 2006;70:453-459.
11. Loumaye A, De Barsy M, Nachit M, et al. Role of activin A and myostatin in human cancer cachexia. *J Clin Endocrinol Metab.* 2015;100:2030-2038.
12. Buehring B, Binkley N. Myostatin - the holy grail for muscle, bone, and fat? *Curr Osteoporos Rep.* 2013;11:407-414.
13. White TA, Lebrasseur NK. Myostatin and sarcopenia: Opportunities and challenges - a mini-review. *Gerontology.* 2014;60:289-293.
14. Miyamoto T, Carrero JJ, Qureshi AR, et al. Circulating follistatin in patients with chronic kidney disease: implications for muscle strength, bone mineral density, inflammation, and survival. *Clin J Am Soc Nephrol.* 2011;6:1001-1008.
15. Berry SD, Ramachandran VS, Cawthon PM, et al. Procollagen type III N-terminal peptide (P3NP) and lean mass: a cross-sectional study. *J Frailty Aging.* 2013;2:129-134.
16. Cavalier E, Delanaye P, Vranken L, et al. Interpretation of serum PTH concentrations with different kits in dialysis patients according to the KDIGO guidelines: importance of the reference (normal) values. *Nephrol Dial Transpl.* 2012;27:1950-1956.
17. Delanaye P, Quinonez K, Buckinx F, Krzesinski J-M, Bruyère O. Hand grip strength measurement in haemodialysis patients: before or after the session? *Clin Kidney J.* 2018:1-4.
18. Roberts HC, Denison HJ, Martin HJ, et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. *Age Ageing.* 2011;40:423-429.
19. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European working Group on sarcopenia in older People. *Age Ageing.* 2010;39:412-423.
20. Rymarz A, Bartoszewicz Z, Szamotulska K, Niemczyk S. The associations between body cell mass and nutritional and inflammatory markers in patients with chronic kidney disease and in subjects without kidney disease. *J Ren Nutr.* 2016;26:87-92.
21. Han DS, Chen YM, Lin SY, et al. Serum myostatin levels and grip strength in normal subjects and patients on maintenance haemodialysis. *Clin Endocrinol (Oxf).* 2011;75:857-863.
22. Qureshi AR, Alvestrand A, Danielsson A, et al. Factors predicting malnutrition in hemodialysis patients: a cross-sectional study. *Kidney Int.* 1998;53:773-782.
23. Besbas N, Ozaltin F, Coşkun T, et al. Relationship of leptin and insulin-like growth factor I to nutritional status in hemodialyzed children. *Pediatr Nephrol.* 2003;18:1255-1259.
24. Jacob V, Le Carpentier JE, Salzano S, et al. IGF-I, a marker of undernutrition in hemodialysis patients. *Am J Clin Nutr.* 1990;52:39-44.
25. Zhang L, Wang XH, Wang H, Du J, Mitch WE. Satellite cell dysfunction and impaired IGF-1 signaling cause CKD-induced muscle atrophy. *J Am Soc Nephrol.* 2010;21:419-427.
26. da Costa JAC, Ikizler TA. Inflammation and insulin resistance as novel mechanisms of wasting in chronic dialysis patients. *Semin Dial.* 2009;22:652-657.
27. Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, Glass DJ. Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. *Am J Physiol Cell Physiol.* 2009;296:C1258-C1270.
28. Cheung WW, Rosengren S, Boyle DL, Mak RH. Modulation of melanocortin signaling ameliorates uremic cachexia. *Kidney Int.* 2008;74:180-186.
29. Schuelke M, Wagner KR, Stolz LE, et al. Myostatin mutation associated with Gross muscle Hypertrophy in a Child. *N Engl J Med.* 2004;351:2682-2688.
30. Breitbart A, Scharf GM, Duncker D, et al. Highly specific detection of myostatin prodomain by an immunoradiometric sandwich assay in serum of healthy individuals and patients. *PLoS One.* 2013;8:e80454.
31. Ju CR, Chen RC. Serum myostatin levels and skeletal muscle wasting in chronic obstructive pulmonary disease. *Respir Med.* 2012;106:102-108.
32. Yarasheski KE, Bhasin S, Sinha-Hikim I, Pak-Loduca J, Gonzalez-Cadavid NF. Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. *J Nutr Health Aging.* 2002;6:343-348.
33. Han DS, Hsiao MY, Wang TG, Chen SY, Yang WS. Association of serum myokines and aerobic exercise training in patients with spinal cord injury: an observational study. *BMC Neurol.* 2016;16:142.
34. Szulc P, Schoppet M, Goettsch C, et al. Endocrine and clinical correlates of myostatin serum concentration in men - the STRAMBO study. *J Clin Endocrinol Metab.* 2012;97:3700-3708.
35. Ma Y, Li X, Zhang H, et al. Serum myostatin in central south Chinese postmenopausal women: relationship with body composition, lipids and bone mineral density. *Endocr Res.* 2016;41:223-228.
36. Lakshman KM, Bhasin S, Corcoran C, et al. Measurement of myostatin concentrations in human serum: Circulating concentrations in young and older men and effects of testosterone administration. *Mol Cell Endocrinol.* 2009;302:26-32.
37. Zachwieja JJ, Smith SR, Sinha-Hikim I, Gonzalez-Cadavid N, Bhasin S. Plasma myostatin-immunoreactive protein is increased after prolonged bed rest with low-dose T3 administration. *J Gravit Physiol.* 1999;6:11-15.
38. Buehring B, Siglinsky E, Krueger D, et al. Comparison of muscle/lean mass measurement methods: correlation with functional and biochemical testing. *Osteoporos Int.* 2018;29:675-683.
39. Burch PM, Pogoryelova O, Palandra J, et al. Reduced serum myostatin concentrations associated with genetic muscle disease progression. *J Neurol.* 2017;264:541-553.
40. Bergen HR, Farr JN, Vanderboom PM, et al. Myostatin as a mediator of sarcopenia versus homeostatic regulator of muscle mass: Insights using a new mass spectrometry-based assay. *Skelet Muscle.* 2015;5:21.
41. Yamada S, Tsuruya K, Yoshida H, et al. Factors associated with the serum myostatin Level in patients Undergoing peritoneal dialysis: potential effects of skeletal muscle mass and Vitamin D receptor activator Use. *Calcif Tissue Int.* 2016;99:13-22.
42. Anaya-Segura MA, García-Martínez FA, Montes-Almanza LÁ, et al. Non-Invasive biomarkers for duchenne muscular dystrophy and Carrier detection. *Molecules.* 2015;20:11154-11172.
43. Han DS, Yu CS, Chiang CK, et al. Serum myostatin is reduced in individuals with metabolic syndrome. *PLoS One.* 2014;9.

44. Carrero JJ, Qureshi AR, Nakashima A, et al. Prevalence and clinical implications of testosterone deficiency in men with end-stage renal disease. *Nephrol Dial Transpl*. 2011;26:184-190.

45. Carrero JJ, Chmielewski M, Axelsson J, et al. Muscle atrophy, inflammation and clinical outcome in incident and prevalent dialysis patients. *Clin Nutr*. 2008;27:557-564.

46. Enoki Y, Watanabe H, Arake R, et al. Indoxyl sulfate potentiates skeletal muscle atrophy by inducing the oxidative stress-mediated expression of myostatin and atrogin-1. *Sci Rep*. 2016;6:32084.

47. Avin KG, Chen NX, Organ JM, et al. Skeletal muscle regeneration and oxidative stress are altered in chronic kidney disease. *PLoS One*. 2016;11:1-15.

48. Guernec A, Chevalier B, Duclos MJ. Nutrient supply enhances both IGF-I and MSTN mRNA levels in chicken skeletal muscle. *Domes Anim Endocrinol*. 2004;26:143-154.

49. Zhang L, Pan J, Dong Y, et al. Stat3 activation links a C/EBPδ to myostatin pathway to stimulate loss of muscle mass. *Cell Metab*. 2013;18:368-379.

50. Saran R, Goel S, Stack A, et al. Serum insulin-like growth factor I levels do not correlate with residual renal function in dialysis patients. *Perit Dial Int*. 2001;21:522-525.

51. Bohé J, Joly MO, Arkouche W, Laville M, Fouque D. Haemodialysis with the biocompatible high permeability AN-69 membrane does not alter plasma insulin-like growth factor-I and insulin-like growth factor binding protein-3. *Nephrol Dial Transpl*. 2001;16:590-594.

52. Ma K, Mallidis C, Bhasin S, et al. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *Am J Physiol Endocrinol Metab*. 2003;285:363-371.

53. Clemmons DR. Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. *Nat Rev Drug Discov*. 2007;6:821-833.