Original Paper

Estimating of Residual Kidney Function by Multi-Frequency Bioelectrical Impedance Analysis in Hemodialysis Patients Without Urine Collection

Ilia Beberashvili^a Tatyana Yermolayeva^b Anna Katkov^a Nedal Garra^a
Leonid Feldman^a Oleg Gorelik^c Kobi Stav^d Shai Efrati^a

^aNephrology Division, Assaf Harofeh Medical Center, Zerifin, affiliated with the Sackler Faculty of Medicine, Tel Aviv University, ^bInternal Department E, Barzilai University Medical Center Campus, Ashkelon, ^cInternal Department F, Assaf Harofeh Medical Center, Zerifin, affiliated with the Sackler Faculty of Medicine, Tel Aviv University, ^dUrology Department, Assaf Harofeh Medical Center, Zerifin, affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Israel

Key Words

Residual kidney function • Hemodialysis • Glomerular filtration rate • Bioimpedance

Abstract

Background/Aims: Residual kidney function (RKF) is a pivotal predictor of better clinical outcomes in maintenance hemodialysis (MHD) patients. So far there has been no attempt to use bioimpedance analysis (BIA) measurements to calculate residual glomerular filtration rate (GFR) in dialysis population. We hypothesized that performing of multi-frequency BIA at the beginning and end of hemodialysis session can enable us to predict the measured residual GFR in MHD patients. Thus our aim was to develop and validate a new RKF prediction equation using multi-frequency BIA in MHD patients. **Methods:** It was diagnostic test evaluation study in a prospective cohort. Participants (n=88; mean age, 66.3±13.2 years, 59.1% males) were recruited from a single hemodialysis center. A new equation (eGFR_{BIA}) to predict RKF, utilizing BIA measurements performed pre- and post-dialysis, was generated and cross-validated by the leave-one-out procedure. GFR estimated as the mean of urea and creatinine clearance (mGFR) using urine collections during entire interdialytic period. **Results:** A prediction equation for mGFR that includes both pre- and post-dialysis BIA measurements provided a better estimate than either pre- or post-dialysis measurements alone. Mean bias between predicted and measured GFR was -0.12 ml/min. Passing and Bablok regression showed no bias and no significant deviation in linearity. Concordance correlation coefficient indicated good agreement between the eGFR_{BIA} and mGFR (0.75, P<0.001). Using cut-off predicted mGFR levels >2 ml/min/1.73 m² yielded an area under curve of 0.96, sensitivity 85%, and specificity

89% in predicting mGFR. The κ scores for intraobserver reproducibility were consistent with substantial agreement between first and second estimation of RKF according to eGFRBIA (weighted κ was 0.60 [0.37-0.83]). **Conclusion:** We present a valid and clinically obtainable method to predict RKF in MHD patients. This method, which uses BIA, may prove as accurate, convenient and easily reproducible while it is operator independent.

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Published by S. Karger AG, Basel

Introduction

Today there is no doubt that residual kidney function (RKF) is a pivotal predictor of better clinical outcomes in maintenance hemodialysis (MHD) patients [1-2]. RKF provides a number of clinical benefits including improved clearance of uremic toxins [3] and hemodynamic stability due to requirement in lower ultrafiltration (UF) rate [4] and contributes consequently to health-related quality of life (QoL), lower inflammation, and significantly less EPO use in MHD population [1]. Decline of RKF during the first year of dialysis has a graded association with all-cause mortality among incident hemodialysis patients [5]. Knowledge of RKF is important not only in terms of prognosis, but in terms of individual adjustment of hemodialysis prescription to RKF level to achieve desirable targets of dialysis adequacy [6], as well as weighing the possibility of “incremental hemodialysis” that has become popular recently [7-8]. A major aim of incremental dialysis is to prevent rapid RKF loss in MHD population [8]. Periodic monitoring of RKF in hemodialysis patients was proposed as a compulsory component of the practical considerations to preserve RKF, especially in incident hemodialysis patients [9].

Since gold-standard measures of kidney function such as inulin clearance or radioisotope clearance techniques are technically difficult and impractical for routine clinical use, alternative methods to estimate residual GFR were developed [6, 10-12]. The European Best Practice Guidelines (EBPG) recommends calculating residual GFR as the mean of creatinine and urea clearance based on interdialytic urine collection, and corrected for body surface area [6]. Still, urine collection during the entire interdialytic period (44 hours) is either uncomfortable or not practical for repeated measurements and is not applicable for MHD patients with low compliance. Recently, poorly dialyzable middle molecules based methods to assess RKF without reliance on urine collections were proposed [10-13]. Equations for calculating residual GFR using Cystatin C [10], β_2 -microglobulin [11-12] and b-trace protein [12] serum concentrations have been developed. However, the use of these biomarkers to date is not common, expensive and may be time consuming. The search for an alternative led us to bioelectrical impedance analysis (BIA) as a good possibility.

Currently, several GFR predicting equations utilizing BIA measurements and anthropometric data are valid for general population [14] and non-dialysis chronic kidney disease (CKD) patients [15-16]. So far there has been no attempt to use BIA measurements to calculate residual GFR in dialysis population. It is well known that RKF may vary over the interdialytic period [6] due to changes in hydration status. On the other hand, the hydration condition also varies during hemodialysis session going from overhydration to euvolemia. The change in hydration status would be less prominent in patients with good RKF. Multi-frequency BIA has been proved as correlated well with these changes in body fluid compartments [17-18]. We hypothesized that performing of multi-frequency BIA at the beginning and end of hemodialysis session can enable us to successfully predict the measured residual GFR in MHD patients. Thus the aim of the present study was to develop and validate a new residual GFR prediction equation using multi-frequency BIA in MHD patients.

Materials and Methods

Study design

This was a single-center cross-sectional study of MHD outpatients receiving dialysis treatment at our institution (Assaf Harofeh Medical Center, Zerifin, Israel). The study was approved by local Institutional Ethics Committee. Informed consent was obtained from all studied patients. Each study participant (in total, 115 MHD patients) was instructed how to collect urine over the entire interdialytic period (starting with empty bladder at the start of one midweek dialysis session [HD1] and ending at the start of the next [HD2]). Blood for urea and creatinine was sampled at the end of the current (we have used the slow flow/stop pump sampling technique) and at the start of the next dialysis session. Residual GFR was measured as the mean of urea and creatinine clearance normalized to 1.73m² body surface area according to European Best Practice Guidelines [6]. Multi-frequency BIA was performed twice - within 30 minutes before and within 30 minutes after the same dialysis session when the blood samples were taken for the serum urea and creatinine at the beginning of session (HD2).

Participants

The study included ESKD patients on regular hemodialysis treatment, who were 18 years or older, reported on the urine production at least 100 ml/24 h and signed a local institutional review board-approved consent form. A flow chart of the study is presented in Fig. 1. Of the 115 patients selected for the study, 9 refused to participate. A total of 106 patients were included, of whom 3 withdrew informed consent. Patients who collected less than 200 ml of urine during the interdialytic period were excluded from the study (n=11). Additionally inaccurate urine collections were excluded (n=4), as decided by the local nurse after interviewing the patient. Of total 88 patients participated in the analysis, 66 patients were randomly selected into a modeling group for derivation of equation for predicting of RKF based on BIA measurements, and the remaining 22 patients were used for validation of the final constructed equation. Further, in 24 patients randomly selected from the study participants, we repeated the blood tests and BIA measurements within two weeks from HD2 to enable a re-evaluation of RKF and to test the reproducibility of the constructed equation in this way.

Test methods

All creatinine and urea measurements in plasma and urine were performed with routine methods. Residual GFR was measured (mGFR) using the following formula [6]:

$$\text{mGFR} = \left[\left(\frac{\text{Uvol}}{t} \right) \times \left(\frac{\text{Uurea}}{\text{Preurea} + \text{Posturea}} \right) \right] + \left[\left(\frac{\text{Ucreat}}{\text{Preact} + \text{Postcreat}} \right) \right] \times (1.73/\text{SA})$$

where t is the urine collection duration (min); Uvol - Urine volume (ml); Preact and Preurea - serum creatinine and urea levels at the start of dialysis session after the completion of urine collection; Postcreat and Posturea - serum creatinine and urea levels at the end of dialysis session before the beginning of urine collection; SA - body surface area (m²).

Body surface area was calculated using Gehan and George formula (19) as follows:

$$\text{SA} = 0.0235 \times \text{Wt}^{0.51456} \times \text{Ht}^{0.42246}$$

where SA is surface area in m²; Wt - weight in kg; Ht - height in cm.

Fig. 1. Flow diagram of the study.

Bioelectric impedance analysis using the multi-frequency body impedance device “Nutriguard-M” (Data-Input, Frankfurt, Germany) was conducted twice - within 30 minutes before and within 30 minutes after the same dialysis session per the study protocol. We used Bianostic AT (Data-Input GmbH) gel-based electrodes specifically developed for BIA measurements. The BIA electrodes were placed on the non-access side of the patient and the patients were in a supine position for at least 5 min before the measurement. Values of resistance (R), reactance (Xc) and phase angle (PhA) were obtained using 3 frequencies: 5, 50 and 100 kHz. Body cell mass, lean body mass, total body water and extracellular water were obtained using the NutriPlus software, version 5.1 (Data Input GmbH, Germany).

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD), or as median with interquartile range (IQR) for variables that did not follow a normal distribution, or as frequencies for categorical data. Normally distributed continuous variables were compared between the two groups using a two-sided t test, with chi-squared tests used for categorical variables and nonparametric Mann-Whitney U tests used for not normally distributed continuous variables.

A new equation was generated using the leave-one-out cross-validation method. Predictive equations for RKF were determined using stepwise linear regression analysis based on BIA measurements performed before and after dialysis session together with potentially significant demographic and clinical parameters. Independent variables were examined for multi-collinearity. All independent variables had a variance inflation factor <3 , suggesting minimal collinearity in the regression models. Residual plots were inspected for normality and homoscedasticity.

The performance of the constructed equation was compared for bias, precision and accuracy. Bias was defined as the median difference between estimated (from constructed equation) GFR (eGFRBIA) and mGFR. Precision was assessed by the standard deviation (SD) of eGFRBIA – mGFR differences; and the Bland-Altman limits of agreement between eGFRBIA and mGFR. Passing and Bablok regression was used to evaluate constant and/or proportional differences between the eGFRBIA and mGFR. Constant differences were evaluated by calculating the intercept of the regression within the 95% CI. Accuracy was assessed by the percentage of GFR estimates that fell within the range of mGFR $\pm 15\%$ (P15) and mGFR $\pm 30\%$ (P30). The concordance correlation coefficient (CCC) and the interclass correlation coefficient (ICC) were calculated to determine the degree of agreement between eGFRBIA and mGFR. The significance of the differences between eGFRBIA and mGFR was determined with the paired sign test for the bias, McNemar’s test for P10 and P30, and a bootstrap method for the SD, ICC and CCC.

Receiving operator characteristic (ROC) analysis was performed for prediction of various cut-off levels of measured GFR using constructed eGFRBIA equation in both modeling and validation groups. Kappa statistics was used in order to assess the reproducibility for the new eGFRBIA equation.

The sample size calculation was based on a significance level of 0.05 with a 90% power to identify a mean difference of 1.0 ml/min between the measured and estimated RKF.

The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS software, version 18.0 (IBM SPSS, Chicago, IL).

Results

Participants

The study cohort consisted of 88 MHD patients. 66 patients were randomly selected into a modeling group and the remaining 22 patients were used for validation of the final constructed equation. There were no significant differences between the modeling and validation groups in terms of age, gender, anthropometric parameters, body composition including lean body mass and hydration status assessed by extracellular water to total body water ratio (Table 1). Serum creatinine and urea concentrations and measured GFR were similar in both groups. Patients in the validation group had a higher interdialytic urine volume than in the modeling cohort.

Table 1. Baseline characteristics of patients. Continuous variables are expressed as mean $\pm$ SD or median with interquartile range (IQR) in case of non-normally distributed data, and categorical variables are expressed as a percentage. Abbreviations: BMI, body mass index; BSA, body surface area; BCM, body cell mass; LBM, lean body mass; ECW/TBW, extracellular water to total body water ratio; GFR, glomerular filtration rate

Descriptive	Modelling group (n = 66)	Validation group (n = 22)	P
Age (y)	65.4 $\pm$ 13.9	69.1 $\pm$ 10.3	0.27
Gender (male %)	60.6	54.5	0.62
Dry weight (kg)	73.9 $\pm$ 16.2	75.4 $\pm$ 13.7	0.70
Height (m)	1.65 $\pm$ 0.08	1.65 $\pm$ 0.10	0.82
BMI (kg/m ²)	27.0 $\pm$ 5.1	27.9 $\pm$ 5.5	0.47
BSA (m ²)	1.85 $\pm$ 0.23	1.87 $\pm$ 0.20	0.72
BCM (kg)	23.3 $\pm$ 6.9	23.5 $\pm$ 5.7	0.90
LBM (kg)	48.6 $\pm$ 9.9	49.9 $\pm$ 10.1	0.47
ECW / TBW	0.39 $\pm$ 0.06	0.40 $\pm$ 0.05	0.47
Predialysis Urea (mg/dl)	115.6 $\pm$ 34.1	110.3 $\pm$ 23.5	0.50
Predialysis Creatinine (mg/dl)	6.45 $\pm$ 2.18	6.51 $\pm$ 1.78	0.90
Interdialytic urine volume (ml)	550 (300-925)	975 (400-1562.5)	0.02
Residual GFR (ml/min/1.73m ²)	2.03 (0.97-3.66)	2.83 (1.04-4.34)	0.41

Table 2. The best linear regression equations for GFR according to modelling group. Abbreviations: BIA, bioelectric impedance analysis; R, resistance, Xc, reactance, PhA, phase angle, H, height

BIA assessment	Parameters	β -coefficient	P-value	Model R ²
Pre-dialysis	Serum creatinine (mg/dl)	1.158	<0.001	0.588
	H2 (m2)	2.595	<0.001	
	PhA50 (°)	1.148	<0.001	
	Xc50-Xc5-Xc100	0.029	0.04	
Post-dialysis	Serum creatinine (mg/dl)	0.846	<0.001	0.649
	H2 (m2)	3.371	<0.001	
	Xc50-Xc100	0.150	0.001	
	Serum creatinine (mg/dl)	0.871	<0.001	
Pre- and post-dialysis	H2 (m2)	4.059	<0.001	0.746
	PhA50post-PhA100post	1.075	0.009	
	R5pre-R100pre	0.037	<0.001	
	Xc50pre-Xc5pre/Xc50post-Xc5post	0.592	0.006	
	Age (y)	0.040	0.01	

Test results

Linear regression models with mGFR as dependent variable were built in the modeling cohort in three different ways: first, using predialysis BIA measurements alone; second, using postdialysis BIA measurements alone and third, using both predialysis and postdialysis BIA measurements. Other relevant covariates were used in each model. The best constructed models are shown in Table 2. The ROC analysis based predictive accuracy of the equation that incorporated both predialysis and postdialysis BIA measurements (model 3) was much better compared with the best model using the only predialysis or postdialysis BIA measurements (model 1 or 2) for cut-off levels 1 to 4 ml/min for mGFR (data not shown). Thus, resulted prediction equation for estimation of mGFR in the modeling group was as follows:

$$eGFR_{BIA} = 1.074 \times (\text{PhA50post} - \text{PhA100post}) + 0.037 \times (\text{R5pre} - \text{R100pre}) - 0.592 \times [(\text{Xc50pre} - \text{Xc5pre}) / (\text{Xc50post} - \text{Xc5post})] + 4.059 \times \text{H2} + 0.040 \times \text{age} - 0.871 \times \text{Cre} - 7.589$$

where eGFR_{BIA} is estimated GFR (ml/min) using BIA measurements; PhA50post and PhA50pre – phase angle at 50 kHz postdialysis and predialysis respectively; R5pre – predialysis resistance at 5 kHz; R100pre – predialysis resistance at 100 kHz; Xc50pre and

Xc5pre – predialysis reactance at 50 kHz and 5 kHz respectively; Xc50post and Xc5post – postdialysis reactance at 50 kHz and 5 kHz respectively; H – height (m); Cre – serum creatinine (mg/dl) predialysis.

The performance characteristics for the constructed equation in estimating mGFR in both modeling and validation groups are shown in Table 3. The median bias of eGFR_{BIA} was negligible and with no statistically significant difference between modeling and validation cohorts. Precision was also similar in the modeling and validation groups (1.52 and 1.54 ml/min/1.73m² respectively). A Bland-Altman analysis revealed that the bias was 0.2 ml/min in the validation group with the limits of agreement ranging from -2.8 to 3.2 ml/min (Fig. 2). The proposed equation provided even better accuracy in validation cohort than in modelling cohort in terms of percentage of eGFR_{BIA} being within 15% or 30% of mGFR. ICC and CCC indicated good agreement between the eGFR_{BIA} and mGFR in both modeling (0.82, P<0.001 and 0.70, P<0.001, respectively) and validation (0.86, P<0.001 and 0.75, P<0.001, respectively) groups. Passing and Bablok regression (Fig. 3) showed no bias and no significant deviation in linearity between the estimated and measured values of GFR. The confidence interval for intercept contains the value 0 and the confidence interval for the slope contains the value 1 in both, the modeling and the validation groups, indicating that both methods have no significant difference and that the eGFR_{BIA} could be used to replace the mGFR.

Table 3. Performance of the new eGFR_{BIA} equation in modelling and validation groups. *p-value by Mann-Whitney U test for bias, by a McNemar test for P15 and P30, and by a bootstrap method for Precision, ICC and CCC. Abbreviations: ICC, intraclass correlation coefficient; CCC, concordance correlation coefficient.

	Modelling group (n = 66)	Validaiton group (n = 22)	P value
Median bias	0.12	0.12	0.75
Precision	1.52	1.54	0.89
Accuracy			
P15 (%)	34.8	40.9	<0.001
P30 (%)	51.5	59.1	0.007
Agreement			
ICC	0.82 (0.71-0.89)	0.86 (0.66-0.94)	0.60
CCC	0.70 (0.55-0.80)	0.75 (0.49-0.89)	0.69

Fig. 2. Bland-Altman plots to compare estimated GFR by BIA (eGFR_{BIA}) and measured GFR (mGFR): (a) modeling group (n=66); (b) validation group (n=22). Mean differences (solid lines) and limits of agreement (dotted lines) are given.

Fig. 3. Comparison of the estimated GFR by BIA (eGFR_{BIA}) versus the measured GFR (mGFR) method by Passing-Bablok regression: (a) modeling group (n=66); intercept with 95% CI: 0.38 (-0.09 to 0.86) and the slope with 95% CI: 0.88 (0.72 to 1.10); Cusum test for linearity – p=0.16; (b) validation group (n=22); intercept with 95% CI: -0.20 (-0.91 to 0.77) and the slope with 95% CI: 1.03 (0.65 to 1.35); Cusum test for linearity – p=0.78. The scatter diagram shows the regression line (solid line), the 95% confidence interval for the regression line (dashed lines) and identity line (x = y, dotted line). The confidence interval for intercept contains the value 0 and the confidence interval for the slope contains the value 1 in both, the modeling and the validation groups. In summary, no bias between the two methods and no significant deviation from linearity could be detected.

Table 4. Receiver operating characteristic analyses for identification of patients with residual GFR above defined levels

Population	N	Cut off residual GFR level to be identified (ml/min/1.73m ²)	AUC	P	Sensitivity at predicted cut off (%)	Specificity at predicted cut off (%)
Modelling	49	>1	0.75	0.002	75	71
	34	>2	0.78	<0.001	74	69
	21	>3	0.82	<0.001	76	73
	12	>4	0.95	<0.001	92	83
Validation	17	>1	0.87	0.01	88	80
	13	>2	0.96	<0.001	85	89
	10	>3	0.90	0.002	90	92
	8	>4	0.92	0.002	86	87

The eGFR_{BIA} equation demonstrated a high degree of accuracy with area under curve values between 0.87 and 0.96 in the validation group (Table 4). Identifying patients in the modeling group with levels of mGFR >2 ml/min/1.73 m², using cut-off predicted mGFR levels >2 ml/min/1.73 m² yielded an area under curve of 0.78, a sensitivity of 74%, and a specificity 69%, while in the validation cohort corresponding values were area under curve 0.96, sensitivity 85%, and specificity 89%.

Repeated measurements of eGFR_{BIA} were carried out within 2 weeks in 24 patients using the constructed equation. The κ scores for intraobserver reproducibility were consistent with substantial agreement between first and second estimation of RKF according to eGFR_{BIA} equation (weighted κ coefficient was 0.60 [0.37-0.83]) (Fig. 4).

To investigate whether residual urine volume might express RKF we compared level of agreement using the kappa statistic (κ) with 95% CI between the proportions of patients with residual urine volume and mGFR and eGFR_{BIA} above cut-off of 2 ml/min/1.73 m². The ROC curve allowed us to define a cut-off value for interdialytic urine output (400ml, i.e. 200ml/day), with a sensitivity of 94% and specificity of 72% for having mGFR ≥2 ml/min/1.73m² (area under the curve, 0.90 [0.56 to 0.80]; P<0.001; data not shown). Thus, residual urine volume was defined as urine volume ≥ 400ml during interdialytic period.

Fig. 4. Box-and-whisker plots of the estimated GFR by BIA (eGFR_{BIA}) and measured GFR (mGFR). Sample size n = 24 each. Each box in the Fig. represents the interquartile range (25th and 75th percentiles); the line inside each box is the median value. The whiskers represent the range of data up to a limit of 1.5-fold above or below the interquartile range. Weighted Kappa coefficient of the intraobserver agreement between the first and the second estimation of GFR by BIA was 0.60 (0.37-0.83).

Fig. 5. Proportion of agreement to disagreement of residual urine volume ≥ 400 ml and mGFR or eGFR_{BIA} ≥ 2 ml/min/1.73m² based on kappa statistic in the modeling and validation groups of patients: a-residual urine volume ≥ 400 ml and mGFR ≥ 2 ml/min/1.73m² in the modeling cohort: $\kappa=0.60$ [0.42 to 0.79]; b-residual urine volume ≥ 400 ml and eGFR_{BIA} ≥ 2 ml/min in the modeling cohort: $\kappa=0.28$ [0.06 to 0.51]; c-residual urine volume ≥ 400 ml and mGFR ≥ 2 ml/min/1.73m² in the validation cohort: $\kappa=0.70$ [0.40 to 1.00]; d-residual urine volume ≥ 400 ml and eGFR_{BIA} ≥ 2 ml/min in the modeling cohort: $\kappa=0.46$ [0.05 to 0.86].

Level of agreement for mGFR above cut-off of 2 ml/min/1.73 m² was good ($\kappa=0.60$ [0.42 to 0.79]) in the modeling cohort as well as in the validation cohort ($\kappa=0.70$ [0.40 to 1.00]) (Fig. 5, a and c respectively). In case of eGFR_{BIA}, level of agreement was fair ($\kappa=0.28$ [0.06 to 0.51]) in the modeling cohort and moderate ($\kappa=0.46$ [0.05 to 0.86]) in the validation cohort (Fig. 5, b and d respectively).

Discussion

In the current study, we developed and tested the validity of an equation using multi-frequency BIA to predict RKF. Our hypothesis that BIA measurements performed at the beginning and the end of the hemodialysis session could be used to predict RKF and that it would perform better than the only predialysis or postdialysis BIA measurements was put to the test. BIA technology allows a specific assessment of the electric properties of tissues that depend on both tissue hydration and structure of CKD patients, including patients undergoing MHD [20]. Fluid overload was found closely associated with the deterioration of RKF in 190 Chinese PD patients [21]. A drastic change in extracellular water during the hemodialysis session accompanied by the fast decline of RKF as well [22]. On the other hand, estimation of excretory renal function in clinical practice is usually based on estimation methods derived from plasma creatinine measurements. According to the recommendations of EBPG group, GFR should be a measure of renal function, rather than renal urea clearance in MHD patients [6]. Plasma creatinine, as an endogenous biomarker of GFR, depends on the level of GFR, the rate of creatinine production (muscle mass) and on its volume of distribution (total body fluids). Besides, it is easily removed during dialysis, therefore, creatinine levels fluctuate significantly between dialysis sessions [13]. From BIA measurements, resistance is inversely related to tissue water content, reactance is proportional to the cell membranes and PhA is an indicator of membrane integrity and water distribution between the intra- and extra-cellular spaces [23]. Taken together, these may explain the rationale for new equation for estimation of RKF: adjustments for BIA measurements (as proxies for hydration and body cell mass), performed before and after dialysis session, improve performance of serum creatinine, predictor demographic and anthropometric variables to predict mGFR in the study population.

On the basis of study by Donadio et al. [14], Flury et al. [16] elaborated and validated an equation incorporating body cell mass (BCM) obtained by BIA to predict creatinine clearance in CKD patients without timed urine collection. Using predicted muscle mass, achieved by BIA measurements, Macdonald et al. [15] improved the prediction of GFR measured by renal inulin clearance in 75 non-diabetic CKD patients. To the best of our knowledge our study is the first to use BIA measurements to predict RKF in MHD patients. Our new equation showed improvements in bias compared to those of Hoek et al. [10], Vilar et al. [11] and Wong et al. [12] equations in their sample. The best constructed regression equation using BIA measurements before and after hemodialysis session could explain 75% of mGFR variance. Overall, our equation showed the good performance in estimating mGFR in the study population— i.e., the low median bias, the high precision, the moderate accuracy and the high CCC. However, limits of agreement are still wide as in middle molecular weight biomarkers based equations for RKF estimation [10-12]. This suggests that our equation may not be accurate enough to estimate RKF. Applying our modeled equation to estimate RKF at cut-off >2 ml/min/1.73 m² in the validation group demonstrated high diagnostic accuracy (85% of sensitivity and 89% of specificity). However, diagnostic accuracy of our equation was less good compared to reported accuracy of the equation based on combined use of b-trace protein and β_2 -microglobulin proposed by Wong et al. [12]. Patients with mGFR >2 ml/min/1.73m² were correctly identified in 100% of cases in the validation cohort with P30 of 77%, but false-positive rate was 13.6%. At the same time, our equation had smaller bias than of that by Wong et al. [12] and high precision in both the modeling and validation

groups. In addition, β 2-microglobulin levels are characterized by a non-specific increase with malignancy and inflammation [13], and can also be affected by age and gender [24]. BIA measurements are, however, not entirely free of these shortcomings, especially PA which may be related to changes in inflammatory mediators [25], and may be affected by age and sex [26] in hemodialysis population. Anyway, our equation based on BIA measurements can be a good alternative to middle molecular weight biomarker-based formulas in hemodialysis patients. Using our equation allows quick result of RKF immediately after performing BIA measurements before and after dialysis, without waiting for laboratory results, and without using expensive laboratory kits. For this purpose, in addition to BIA measurements, it is enough to know the height of the patient, age and the level of plasma creatinine. Additionally, laboratory standards for beta-trace protein and cystatin C to ensure consistency in inter- and intra-laboratory measurements not yet established [27-28].

Our prediction equation was developed using BIA and RKF measurements at a single time point. Because RKF is lost over time, repeated measurements are needed and therefore a question of reproducibility of the method is very relational. Variability of BIA measurements at the same patient at different points of time can lead to different estimations of RKF by our equation. For example, a coefficient of variation for bioelectrical impedance parameters in end-stage kidney disease patients tested on two occasions separated by 3 months ranged from 4.1 to 7.4% [29]. Our study demonstrated substantial intraobserver agreement for the eGFRBIA repeated within 2 weeks interval between the measurements with the assumption that renal function should not change during this period. Therefore, RKF estimation by our equation is reproducible and can serve for repeated measurements.

In our study, we excluded anuric patients but included MHD patients with low level urine volume still allowing calculating mGFR based on interdialytic urine collection. In our opinion it was essential to include such patients to understand the limitations of using multi-frequency BIA to predict very low level RKF. Excluding such patients would have limited us to predict very low level kidney function.

A last question we tried to touch was whether residual urine volume is good enough to characterize the RKF and to what extent it corresponds to mGFR and/or eGFRBIA in this regard. Residual urine volume was even more closely associated with adverse outcomes compared with the other RKF indices in a recent study [2]. Rely on our statistical analysis residual urine volume showed good reliability to express mGFR and substantial reliability to express eGFRBIA. However, it is somewhat tricky to correctly interpret these data because residual urine volume is incorporated in the mGFR calculation equation and mathematical coupling can mislead. It is therefore advisable to compare it with an independent test, ideally with gold standard test (such as radionuclide techniques).

Our study has several limitations. First, the number of patients in validation group was relatively small (n=22) and our modeled equation based on a small sample size that may limit its applicability in wide MHD population with various co-morbidities. However, we generated predictive equation for RKF principally to test the hypothesis that including BIA measurements performed at the beginning and end of hemodialysis session will enable RKF estimation. While we provide the new equation to be used in its present form, it requires generation from a larger data set and full validation of the performance in multiple centers before being used in clinical practice. Second, our equation for predicting of RKF should be evaluated against the gold standard for mGFR, such as radionuclide techniques. We have used the mean of urea and creatinine clearance using urine collections during interdialytic period, because this is a commonly used method for calculating GFR in MHD [6]. Significant interlaboratory variability in creatinine measurement [30] which may influence the performance of our equation is also a subject for consideration. Third, neither a modelling group nor a validation groups were included patients with underweight (BMI<20kg/m²) and severe obesity (BMI>35kg/m²). Since BIA prediction errors appear to be important in patients with severe obesity [17] or in female subjects with underweight [31], our RKF prediction formula cannot be extended to these patients.

Conclusion

We present a valid and clinically obtainable method to predict RKF in MHD patients. This method, which uses BIA, may prove as accurate, convenient and easily reproducible while it is operator independent. Inclusion of both, pre- and post-dialysis BIA measurements into regression equations can provide a better estimate of RKF than either pre- or post-dialysis measurement alone. However, it should be used with caution in patients with low levels of RKF. Our equation may be more useful clinically to identify those with significant RKF (mGFR > 2 ml/min/1.73 m² BSA), recognizing that sensitivity of this test is limited. Further development and validation of this method is now required before implementation in clinical practice.

Disclosure Statement

The authors declare they have no Disclosure Statement.

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