Kidney International, Vol. 46 (1994), pp. 534–539

TECHNICAL NOTE

A new method for monitoring body fluid variation by bioimpedance analysis: The RXc graph

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Bioelectrical impedance analysis (BIA) is a safe, noninvasive, rapid, reproducible, portable and inexpensive method using simple equations for safely and accurately estimating the total body water with a correlation coefficient of 0.996, and 1.67 kg standard error of estimate [1, 2]. BIA-derived equations, validated in a healthy population, produce biased estimates of compartment volumes in patients with fluid overloading, or with dehydration, since formulae are necessarily bound to normal body weight and composition [3]. Due to the shape of the pressure-volume curve of the interstitial fluid spaces, edema is not usually detectable until the interstitial fluid volume has risen to about 30% (4 to 5 liters) above normal [4]. The literature does not provide any cut-off value for BIA measurements in the bedside identification of the individual patient's fluid status [3], either with fluid overload before the appearance of edema or with dehydration before clinical signs. However, both components of the impedance vector Z, namely resistance (R) and reactance (Xc), separately considered, correlated highly with net fluid balance in cardiac patients after surgery [5], and in the rehydration of cholera patients [6].

In this study, we present a new approach for routine monitoring of the body fluid variation in the single patient, without making any assumption on body composition. The method is based on the analysis of the bivariate distribution of the impedance vector in a healthy population and in patients with increased body weight, due to either obesity or edema from renal diseases.

Methods

To validate the new method, we studied a total of 217 adult caucasian subjects who gave informed consent. Subjects were divided into four classification groups: 86 healthy control subjects (CS) (38 males, 48 females, age 16 to 66 years), 55 patients (31 males, 24 females, age 18 to 75 years) with mild to terminal chronic renal failure (CRF) in conservative treatment (serum creatinine 124 to 1912 μmol/liter (1.4 to 21.6 mg/dl)), with undetectable to severe edema (15% with apparent edema), 36 patients (19 males, 17 females, age 16 to 75 years) with idiopathic nephrotic syndrome (NS) (proteinuria from 3.5 to 23 g/day; 28% in the interval 3.5 to 5 g/day, 39% in the interval 5 to 10 g/day, and 33% in the interval 10 to 23 g/day), with undetectable to severe edema (58% with apparent edema), and 40 obese subjects (OS) (9 males, 31 females, age 24 to 71 years) with body mass index (BMI) higher than 31 kg/m^2, free from diabetes, kidney, heart and liver diseases.

All subjects had height (H), weight, BMI, and BIA determined on the same day. Serum creatinine, total protein, albumin, Na, and urinary protein concentrations were determined according to standard laboratory methods. Oncotic pressure was estimated from serum total protein and albumin concentrations according to Miller and Meyer [7]. BIA was performed with an impedance plethysmograph, which emitted 800 μA and 50 kHz alternating sinusoidal current (model BIA-109 RJL/Akern Systems, Detroit, Michigan, USA) and was connected to surface electrodes (standard, tetrapolar placement on the right hand and foot) strictly following the method reported elsewhere [5, 6, 8]. The two components of the whole-body impedance vector, R and Xc, were recorded from single representative stable measurements, conducted by the same operator. We standardized BIA measurements by the H of subjects, thus expressing both R/H and Xc/H in Ohm/m. The mean coefficient of variation was 1% for within-day and 3% for weekly intraindividual measurements in the steady state condition, and 2% for the interoperator variability (1 to 2 cm displacement of electrodes from the anatomical reference points).

Differences in mean values of protocol variables among groups were assessed by the two-way analysis of variance (ANOVA, F test), considering sex and disease groups as classification criteria. Relationships between variables were assessed by linear correlation analysis (r coefficient). The programs of the statistical package BMDP [9] were used for calculations.

Assuming the bivariate normal distribution [10] of R/H and Xc/H, we calculated the bivariate 95% confidence limits (**Appendix**) for mean impedance vectors of the different classification groups (that is, the ellipse, within which the two-dimensional mean vectors fall with a 95% probability; Fig. 1). We called the "RXc mean graph" the average of R/H and Xc/H, recorded in groups of patients and plotted on the 95% confidence ellipse for the healthy population (Figs. 1 and 2). For the evaluation of the individual patient's vector, we calculated the bivariate 75% and 95% tolerance limits (**Appendix**) of the reference vector (that is, the ellipses for the healthy population, within which the vector of the individual subject falls with a probability of 75% and 95%, respectively; Fig. 3). The "RXc point graph" was defined as a single measurement of the two height-standardized components of the impedance vector, namely R/H and Xc/H, recorded in the individual patient and plotted on the reference, 75% and 95%

Received for publication December 14, 1993
and in revised form March 14, 1994
Accepted for publication March 14, 1994

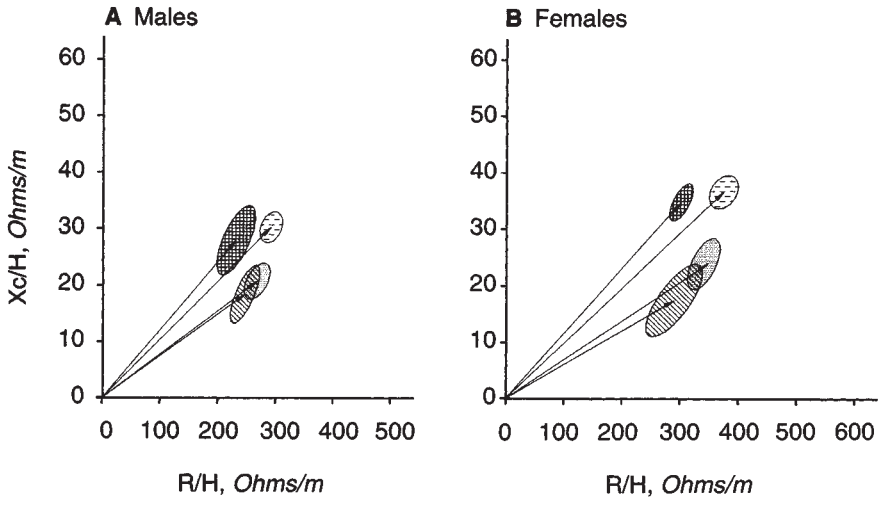

Fig. 1. *"RXc mean graph" with the 95% confidence ellipses for males (A) and females (B) of the classification groups.* Symbols are: (⊟) CS, healthy control subjects, (▨) CRF, chronic renal failure, (▧) NS, nephrotic syndrome, (▩) OS, obese subjects). R is the resistance, Xc the reactance, and H the height.

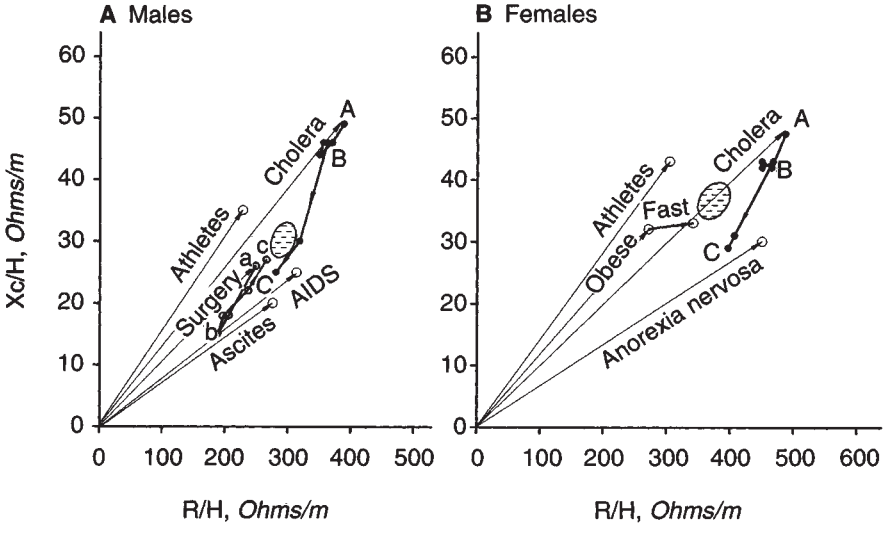

Fig. 2. *"RXc mean graph" with data drawn from literature and plotted over the sex-specific 95% confidence ellipse for our healthy population.* Left panel vectors are: athletes [17], cholera [6] [admission with dehydration (A), first 24 hours of rehydration (B), 10th day hydration (C)], surgery [5] [leaving the preoperative day (a), fluid infusions moved the vector back on the first postoperative day (b), and on the 7th day the mean vector returned to the baseline (c)], AIDS [18], and ascites [14]. Right panel vectors are: athletes [17], obese before and after short-term fasting [16], cholera [6] (as in left panel) and anorexia nervosa [19]. R is the resistance, Xc the reactance, and H the height.

tolerance ellipses for the healthy population (Fig. 3). The "RXc path graph" was defined as the walk of the successive point measurements of the impedance vector, recorded over a follow-up period in the same subject, and plotted over the reference 75% and 95% tolerance ellipses for the healthy population (Fig. 4).

Results

Results considering the disease group and gender are reported in Table 1 and Figure 1. With respect to the CS group vector, a shorter impedance vector was demonstrated in both obese and renal patients, without any overlapping of confidence ellipses (that is, average vectors with a phase angle significantly greater in OS and significantly smaller in renal patients; Fig. 1). The 95% confidence ellipses of the two renal patient groups were overlapping in both males and females (that is, no significant vector displacement).

The two components of the impedance vector, R/H and Xc/H, were significantly and linearly correlated in all groups considered, either as a whole or by sex, with apparently lower r values in the CS group (Table 2), which was reflected by the shape (relation between the major and minor axis) of confidence and tolerance ellipses (Figs. 1 and 3, respectively). R/H and Xc/H were not significantly correlated with age in the CS group, as a whole or by sex (absolute r values less than 0.15 and 0.23, respectively). Therefore, we did not plot the set of (overlapping) tolerance ellipses by sex and age classes for the reference impedance vector. We found that disease and sex had a significant effect on the means of both R/H and Xc/H (Table 1) using the conventional univariate analysis (two-way ANOVA). There was no significant interaction between sex and disease classification for R/H nor for Xc/H (Table 1). The same results were found considering non-standardized R and Xc measurements (data not reported).

To find operative cut-off percentiles of the bivariate distribution for the impedance vector, we plotted the impedance vector of single patients over the sex-specific reference tolerance ellipses (95th and 75th percentile) for the healthy population (Fig. 3). No obese subject fell out of the 95% tolerance ellipse. No renal patient fell out of the upper poles of either 95% and 75% tolerance ellipses, while 29 (32%) out of the 91 renal patients fell out of the lower pole of the reference 95% tolerance ellipse.

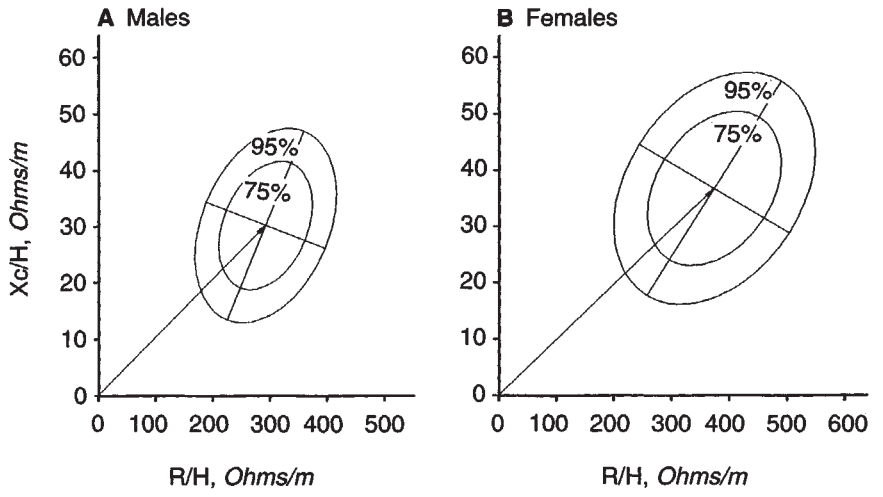

Fig. 3. *"RXc point graph" with the vector of the average healthy subject plotted over the reference, sex-specific 75% and 95% tolerance ellipses of the healthy population.* **A.** Males; **B.** females. R is the resistance, Xc the reactance, and H the height.

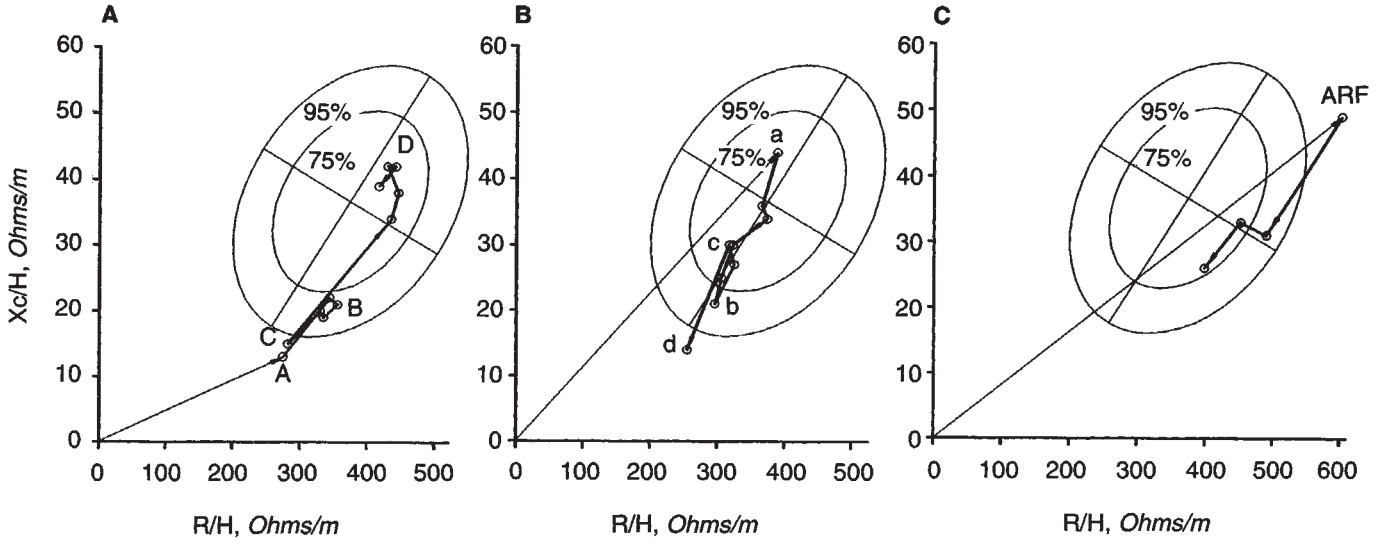

Fig. 4. *"RXc path graph" of three women.* The shortest vector (left panel) was measured in a nephrotic patient with edema (A), undergoing remission of proteinuria (B), with a subsequent relapse, due to tapering of the steroid (C), finally reaching her normal body composition after stable, complete remission of proteinuria and full recovery of physical activity (D). The middle vector (middle panel) was measured in a patient with mild chronic renal insufficiency, normal metabolic profile, body composition, and physical activity (a). Progressive renal failure caused a fluid overload (b), at first controlled by diuretics (c), but finally requiring starting of chronic dialysis (d). The longest vector (right panel) was measured in a patient with acute renal failure (ARF), caused by fluid loss. Renal function was restored after the impedance vector crossed back over the 75% tolerance ellipse, due to large fluid infusion.

Moreover, 56 (62%) out of 91 renal patients fell out of the lower pole of the 75% tolerance ellipse. Twenty-nine (32%) out of the 91 renal patients had apparent edema at the time of BIA measurements. Twenty-eight (97%) of the 29 patients with apparent edema fell out of the lower pole of the 75% tolerance ellipse, of which 20 (69%) fell out of the lower pole of the 95% tolerance ellipse. The fluid overload was heavier in those with shorter down-sloping impedance vectors. One patient with apparent edema fell within the lower pole of the 75% tolerance ellipse. Seventeen (20%) of the 86 CS and 7 (18%) of the 40 OS fell out of the 75% tolerance ellipse. Of these, 6 (7%) CS and 4 (10%) OS fell out of the lower half of the 75% tolerance ellipse. Thus, the lower pole of the sex-specific 75% tolerance ellipse was considered a bioelectrical impedance threshold for apparent edema in renal patients.

In CRF patients, serum creatinine levels did not significantly correlate with either R/H ($r = -0.18$) or Xc/H ($r = -0.21$). Plasma Na concentration was not correlated with either R/H ($r = 0.02$) or Xc/H ($r = 0.11$) in the CRF group. In the NS group it was significantly correlated with R/H ($r = 0.37$, $P = 0.03$), but not with Xc/H ($r = 0.22$). In NS patients, plasma oncotic pressure was linearly and significantly correlated with Xc/H ($r = 0.53$, $P < 0.001$), but not with R/H ($r = 0.31$). In CRF patients, oncotic pressure did not correlate with either Xc/H or R/H ($r = 0.20$, and $r = 0.01$, respectively). Therefore, despite significant correlations, the protocol variables possibly involved in extracellular fluid

Table 1. Mean values with standard error (SEM), reported by sex and disease groups

	CS		CRF		NS		OS				
Groups	Males	Females	Males	Females	Males	Females	Males	Females			
N	38	48	31	24	19	17	9	31	F_D	F_{Sex}	F_{D*sex}
R/H, *Ohm/m*											
mean	292.6	374.3	268.1	340.4	247.2	289.7	231.6	301.5	18.3[b]	65.5[b]	1.1
SEM	7.6	9.7	8.7	10.5	9.4	17.2	10.2	7.7			
Xc/H, *Ohm/m*											
mean	30.2	36.6	20.7	23.9	18.3	17.5	27.9	34.8	47.7[b]	11.6[b]	2.4
SEM	1.1	1.2	1.2	1.7	1.9	2.3	1.9	1.2			
BMI, kg/m^2											
mean	24.5	24.8	24.2	23.5	25.0	27.2	36.6	35.7	77.2[b]	0.1	1.3
SEM	0.5	0.5	0.4	0.9	0.8	1.0	1.7	0.8			
Height, *cm*											
mean	176.2	161.9	171.9	160.5	172.7	161.9	173.4	158.2	2.8[a]	153.8[b]	1.0
SEM	1.1	1.0	1.1	1.4	1.5	1.9	2.6	1.1			

Abbreviations are: CS, healthy control subjects; CRF, chronic renal failure; NS, nephrotic syndrome; OS, obese subjects; R/H, resistance/height; Xc/H, reactance/height.

F_D, F_{Sex}, and F_{D*sex} are F tests of the two-way ANOVA, for disease (D), sex and interaction effect.

[a] $P < 0.05$

[b] $P < 0.0001$

Table 2. Linear correlation coefficient (r) between the components resistance/height (R/H) and reactance/height (Xc/H) of the impedance vector

Groups		CS	CRF	NS	OS
Whole group	*N*	86	55	36	40
	r	0.477	0.556	0.667	0.712
	P	< 0.001	< 0.001	< 0.001	< 0.001
Males	*N*	38	31	19	9
	r	0.323	0.508	0.736	0.678
	P	0.048	0.004	< 0.001	0.045
Females	*N*	48	24	17	31
	r	0.333	0.586	0.756	0.637
	P	0.021	0.003	< 0.001	< 0.001

Values are given by sex and the following disease groups: CS, control subjects; CRF, chronic renal failure; NS, nephrotic syndrome; OS, obese subjects.

regulation accounted for less than 28% of the variability of either impedance vector components, as indicated by the r^2 values.

Discussion

When the aim is to compare the mean impedance vector of groups of subjects, methods using the bivariate confidence intervals are appropriate [10]. Plotting data on a "RXc mean graph" allows statistical testing and also easy interpretation of different vector displacements (Figs. 1 and 2), since both the direction (that is, the phase angle) and the magnitude of the impedance vector are simultaneously considered. We could demonstrate a significantly shorter impedance vector in both obese and renal patients with respect to the healthy population (Fig. 1). Due to the difference in the phase angle, BIA could discriminate between obese and renal patients, but not between NS and CRF vector displacements (Fig. 1). Because of the mutual correlation between R and Xc, we are cautious in accepting that either individual components of the impedance vector reflect changes in specific fluid compartments [3, 11, 12]. We could not draw the confidence ellipse for healthy populations reported in literature since neither Xc measurements nor correlation between R and Xc have been documented by gender. However, similar average values for both R and Xc in distinctly different populations, that is, in a large Italian population from a different region [13] and in Peruvians, have been reported [6]. Data drawn from literature using the same BIA technique and transformed onto the "RXc mean graph" suggest that other clinical conditions characterized by fluid overload, such as cardiac surgery [5] and cirrhosis with ascites [14], are characterized by average vector displacements as in renal patients (Fig. 2).

To directly evaluate the fluid status of the individual patient by BIA measurements, we must refer to the bivariate tolerance intervals (tolerance ellipses) and thus use the confidence intervals of mean vectors (confidence ellipses) as directional trends for the interpretation of the single impedance vectors. Sex-specific tolerance ellipses (Fig. 3) appeared smaller in males than in females, but still covered a large region of the RXc plane. This difference can be accounted for by a greater variability in body composition of either pre- or post-menopausal females over the wide age range considered. However, when the aim is to compare the single impedance vector of an individual subject with a reference population, tolerance ellipses are required. Our "RXc point graph" which uses tolerance ellipses corresponding to clinically meaningful cut-off percentiles, allows straightforward interpretation of impedance vector placement. Since the vectors of renal patients covered a plane region less than 1/5 of the tolerance ellipses, close to the lower poles, less than 1% and 5% of the healthy population were expected (and observed) to overlap out of the lower poles of the 95% and 75% tolerance ellipses, respectively (**Appendix**). However, the probability distribution calculated on larger samples could be represented by smaller ellipses, and populations of different races might yield different shape and sizes for sex-specific ellipses.

Monitoring of fluid overloading or removal in the single patient is immediate with the "RXc path graph." It allows direct comparison of intrasubject (analytical, physiological and pathological components) with intersubject variability. As depicted in Figure 4, while a progressive shortening and down-sloping of the impedance vector over successive measurements indicates progressive fluid overloading, a progressive lengthening and steepening of the

vector indicates fluid removal. A body fluid variation in both directions in the same patient causes a symmetric backward and forward shift of the vector on the RXc plane (Fig. 4). Therefore, the aim of therapy is to bring the individual vector back into the 75% tolerance ellipse towards the reference target point (that is, the crossing of the reference, sex-and-race specific ellipse axes), or better to a previously recorded healthy target point for that patient. As a working hypothesis, we believe that a progressive shortening and down-sloping of the impedance vector could correspond to a progressive shift to the right of the interstitial pressure-volume curve [4], whose sudden increase in the slope (positive pressure with the appearance of free fluid and edema) could occur when the impedance vector falls out of the lower pole of the 75% tolerance ellipse. The intra-subject variation of the interstitial negative pressure (gel hydration) could be described by impedance vector displacement within the 75% tolerance ellipse before clinically detectable fluid volume variation. The impedance vectors of patients falling out of the upper 95% and 75% poles of the tolerance ellipses indicate dehydration (Fig. 4, ARF vector), also clearly shown after the transformation of the measurements obtained in cholera dehydration [6] onto the 'RXc mean graph" (Fig. 2). Since our study was designed to find BIA thresholds for fluid overload, the relationship between BIA and dehydration requires further investigation.

Most obese patients fell within the left half of the 75% tolerance ellipse. Interestingly, an increased ratio of extracellular to intracellular water in obesity [15] and mean impedance values for obese females close to ours [16] have been reported in literature (Fig. 2). Extremes of lean body mass such as in athletes [17] or wasting conditions, such as AIDS [18] and anorexia nervosa [19], do not show a shortening of vectors with respect to CS group, but a displacement either upward or downward, respectively (Fig. 2). These observations can open new horizons for the use of the RXc graphs in monitoring the body composition and the nutritional status of patients with or without fluid disorders [20].

We used the conventional, whole-body BIA measurement (tetrapolar, with 50 kHz stimulation frequency) which reflects total body water with the best accuracy required for clinical evaluation and with a greater history of validation [1–3, 5, 6, 8, 11, 15, 21–25], but the RXc graph method might yield better results with different BIA techniques, in particular combining multifrequency with different electrodes placements [26–28]. However, following the traditional BIA approach, where the accuracy of formulae predicting the volume of total body water and the fat-free mass is significantly improved by the inclusion of variables such as weight, age, and sex in addition to the impedance index H^2/R [1, 2], the user should be familiar with the equation supplied by the manufacturer of the impedance analyzer and know which "gold standard" body composition technique and reference population were used to generate the equation. Operating with the RXc graphs only requires plotting direct R/H and Xc/H measurements on the reference population distribution, which allows one to decide whether a patient has maintained normal hydration forfeiting prediction of total body water in liters. If the former estimates of volume and mass are of interest, and the patient's impedance vector lies within the 75% tolerance ellipse, the traditional prediction formulae can be applied with confidence.

Until now we have considered steady-state conditions but pilot studies indicate a useful application of our RXc graph method in dynamic situations as well, when fluid is removed (dialysis, and extracorporeal ultrafiltration) or infused (hydration and total parenteral nutrition). Indeed, rigorous studies are necessary to establish the correspondence between impedance vector displacement over the RXc plane and subtle body fluid variation. The greatest potential use of the method might be in those patients in whom baseline impedance vectors (at least 10 points, to achieve reasonable tolerance intervals) have already been measured in the healthy condition, so that subsequent changes away from their own smaller tolerance ellipses might be significant even if they remained within the reference population 75% tolerance ellipse.

In conclusion, we found a bioimpedance threshold for fluid overload and a useful innovative graphical method for identification, monitoring and therapy planning of renal patients with altered fluid balance using direct bioimpedance measurements and following a new bivariate vectorial approach. Further rigorous studies are necessary to define whether the method proves to be generally applicable to a variety of different clinical situations.

Acknowledgments

This work was supported by the National Research Council [(CNR)-Targeted Project "Prevention and Control of Disease Factors", Subproject "Feeding"], grant 93.00598.PF41.

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Appendix

For readers less familiar with multivariate analysis, we report the basic definitions of multivariate confidence and tolerance intervals as well as formulae for their calculations in the particular case of the assumption of the bivariate normal distribution for the RXc graph.

Confidence interval is the inferential statistical interval for a given parameter (such as a mean value) investigated. It is the region in the parameter space to which is assigned the probability $100(1 - \alpha)\%$ (α is some fixed probability, typically 0.05) that the parameter vector lies within. The confidence interval of the mean of the univariate normal distribution is formed by two values (limits), while the interval of the mean vector of the multinormal distribution is an ellipsoid centered at the mean vector, which reduces to a hypersphere when the correlation coefficients between pairs of variables are zero. When the confidence ellipsoids of two mean vectors overlap, the null hypothesis of equality of the two mean vectors cannot be rejected with the significance level α. The 95% confidence ellipses of several impedance mean (bivariate) vectors are depicted in Figure 1.

Tolerance interval is the probability interval within which a specified proportion of a distribution (such as a population) will lie with a fixed probability $100(1 - \alpha)\%$. The α values are selected for the particular descriptive or inferential purposes, typically ranging from 0.5 (median), 0.75 (third quartile), to 0.95 or 0.99 (95th or 99th percentiles). Such interval can be used to decide whether particular observations (individual vectors) are from the same population as a previous sample, used to determine the interval. The tolerance intervals of the multinormal distribution are ellipsoids (contours of equal concentrations centered at the mean vector), which reduce to hyperspheres when the correlation coefficients between pairs of variables are zero. Figure 3 depicts the 75% and 95% tolerance ellipses for our healthy population. Since 25% and 5% of observations are expected to lie out of the 75% and 95% tolerance ellipses, respectively, 6.3% and 1.2% are expected to lie out of the contour of the four ellipses' quadrants, and 5% and 1% will lie out of a 1/5 partition of the ellipses' contour (for example, the lower pole).

Both the confidence and tolerance intervals become smaller with increasing sample size. But, in a very large population (such as multinormal), while the confidence interval converges to the parameter vector (for example, the mean vector point), the tolerance interval converges to the interval corresponding to the quantiles of the population within which lies the percentage of the (infinite) population to which the tolerance interval relates (such as the ellipsoid for the particular α value).

Calculations. In the case of the bivariate normal distribution, both approximate and exact methods are available for calculations of both confidence and tolerance ellipses [29]. We present our modified version of the exact methods using common statistics of the simple linear correlation analysis. Given n pairs of observations x and y, with standard deviations s_x and s_y, and correlation coefficient r, one must fix the α probability level and take the Snedecor's F_α value with 2 and n-2 degrees of freedom. The semi-axes L_1 and L_2, and the slopes b_1 and $b_2 = -1/b_1$, of the axes of the $100(1 - \alpha)\%$ confidence and tolerance ellipses can be calculated using equations (1) and (2), respectively.

$$L_1, L_2 = K\sqrt{(n-1)(s_x^2 + s_y^2) \pm \sqrt{[(n-1)(s_x^2 + s_y^2)]^2 - 4(n-1)^2(1 - r^2)s_x^2 s_y^2}} \quad \text{(Eq. 1)}$$

where

$K = F/n(n - 2)$ for confidence ellipses

$K = F(n + 1)/n(n - 2)$ for tolerance ellipses

$$b_1 - 1/b = (s_y^2 - s_x^2)/2rs_xs_y \pm \sqrt{1 + [(s_y^2 - s_x^2)/2rs_xs_y]^2} \quad \text{(Eq. 2)}$$

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