Original article

Nuclear Medicine Communications

Simplified methods for assessment of renal function as the ratio of glomerular filtration rate to extracellular fluid volume

Lars Jødal and Jens Brøchner-Mortensen

Background **Instead of scaling glomerular filtration rate (GFR) to a body surface area of 1.73 m^2, it has been suggested to scale GFR to extracellular fluid volume (ECV). The ratio GFR/ECV has physiological meaning in that it indicates how often 'that which is to be regulated' (i.e. ECV) comes into contact with the 'regulator' (i.e. the kidneys).**

Aim **The aim of the present study was as follows: to analyse two published calculation methods for determining ECV and GFR/ECV; to develop a new simple and accurate formula for determining ECV; and to compare and evaluate these methods.**

Materials and methods **GFR was determined as ^{51}Cr-EDTA clearance. The study comprised 128 individuals (35 women, 66 men and 27 children) with a full ^{51}Cr-EDTA plasma concentration curve, determined from injection until 4–5 h p.i. Reference values for GFR and ECV were calculated from the full curve. One-pool approximations Cl_1 and V_1 were calculated using only the final-slope curve. Four calculation methods were compared: simple one-pool values; GFR/ECV according to Peters and colleagues; ECV according to Brøchner-Mortensen (BM); and ECV according to a new method (JBM): $y = 2x - 1$, where $x = Cl_1/Cl$ and $y = V_1/ECV$.**

Results **The new JBM method is accurate and can be explained theoretically. BM has a slight bias for high renal function. The Peters method had bias in our data. GFR/ECV had better precision than ECV alone, especially for BM and JBM, which were within −4% to +7% of the reference values (95% limits of agreement in adults).**

Conclusion **GFR/ECV can be precisely determined, especially with the BM and JBM methods. Expressing GFR/ECV in unit %/h gives a simple interpretation. Normal ranges for GFR/ECV need to be established.** ***Nucl Med Commun*** **33:1243–1253** © **2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Nuclear Medicine Communications 2012, **33**:1243–1253

Keywords: ^{51}**Cr-EDTA, extracellular fluid volume, glomerular filtration rate, renal function**

Department of Nuclear Medicine, Aalborg University Hospital, Aalborg, Denmark

Correspondence to Lars Jødal, MSc, Department of Nuclear Medicine, Aalborg University Hospital, P.O. Box 365, DK-9100 Aalborg, Denmark
Tel: +45 9932 3139; fax: +45 9932 3145; e-mail: lajo@rn.dk

Received 14 February 2012 **Revised** 24 May 2012 **Accepted** 2 August 2012

Introduction

The glomerular filtration rate (GFR) is the most widely used single parameter for assessing renal function. Most often, GFR is expressed in relation to a standard body surface area (BSA) of 1.73 m^2. The normalization to BSA has long historical roots [1] but has no physiological explanation and may, for instance, underestimate renal function in very obese individuals [2].

As an alternative, it has been suggested to scale GFR to extracellular fluid volume (ECV) [3,4]. Unlike surface-normalized GFR, the ratio GFR/ECV (or the reverse ratio ECV/GFR) has a direct physiological interpretation. As expressed in Brøchner-Mortensen [3]:

> These easily determined ratios indicate how often 'that which is to be regulated' (i.e. the extracellular fluids) comes into contact with 'the regulator' (i.e. the kidney).

A common technique for GFR determination is bolus injection of a GFR marker (^{51}Cr-EDTA, ^{99m}Tc-DTPA, or iohexol), followed by the taking of two or more blood samples 3–4 h after the injection. However, it is much less commonly recognized that ECV and the ratio GFR/ECV can be determined from the same data. Brøchner-Mortensen [3] described how ECV could be calculated from the slope and intercept of the final part of the plasma concentration curve. The ratio GFR/ECV can be calculated afterwards. Interestingly, his data indicated that the precision of the ratio was in fact better than the precision of ECV alone. Peters [4,5] determined the ratio of GFR/ECV directly from the slope.

The aim of the present study is three-fold: (i) to evaluate the approaches of Peters and of Brøchner-Mortensen; (ii) to develop a new simple and accurate formula for determination of ECV and thereby the ratio GFR/ECV; and (iii) to evaluate and compare these approaches.

Materials and methods

Overview of calculations

Clearance of ^{51}Cr-EDTA determined from the full plasma curve was used as the reference value for the glomerular filtration rate, GFR_{ref}. (This choice is further discussed in the Discussion section.) Similarly, the distribution volume for ^{51}Cr-EDTA was used as the reference value for the extracellular fluid volume, ECV_{ref}. Symbols and methods have been summarized in Tables 1 and 2. Details are found in the Calculations section.

0143-3636 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

DOI: 10.1097/MNM.0b013e3283591908

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

For all the methods tested, differences from reference values were computed as follows:

$$\text{Absolute difference} = \text{Method value} - \text{Reference value},$$

$$\text{Relative difference} = \frac{\text{Absolute difference}}{\text{Reference value}} \times 100\,\%.$$

Bias and SD were computed corresponding to the mean and SD of the relative differences (for details, see the Calculations section).

Table 1 Summary of symbols

Symbol	Explanation
b_1	Rate constant (slope) of the slowest exponential in the plasma concentration curve[a]
c_1	Intercept of the slowest exponential in the plasma concentration curve[b]
Cl	Clearance (determined from the full plasma concentration curve)
Cl_1	One-pool clearance, calculated from only the slowest exponential in the plasma concentration curve (also called slope–intercept clearance)
ECV	Extracellular fluid volume = distribution volume of the tracer
V_1	One-pool volume, calculated from only the slowest exponential

[a]Most often denoted as α_2 by Peters [5], who normally uses two exponentials for the full plasma concentration curve.
[b]Denoted as B by Peters, see the note above.

Table 2 Reference methods and compared methods

Definition	Determination	Equation
Reference methods		
GFR_{ref}	^{51}Cr-EDTA plasma clearance, Cl_{EDTA}, determined from the full plasma curve	Eq. (2)
ECV_{ref}	Distribution volume of ^{51}Cr-EDTA. Determined from the full plasma curve as the product of Cl_{EDTA} and mean transit time in the body	Eq. (6)
Simple slope-only method (Peters' method before correction)		
$(GFR/ECV)_{simple}$	Calculated as the rate constant of the slowest exponential = slope (b_1)	Eq. (13)
Simple volume estimate		
$ECV_{simple} = V_1$	Distribution volume from the slope (b_1) and intercept (c_1) of the slowest exponential	Eq. (9)
Peters' method with correction		
$(GFR/ECV)_{Peters}$	Calculated as $b_1 + 15.4{b_1}^2$ [5]	Eq. (14)
ECV_{Peters}	Calculated as $GFR_{JBM}/(GFR/ECV)_{Peters}$	Eq. (15)
Brøchner-Mortensen (BM)		
ECV_{BM}	Calculated from Cl_1, b_1 and Cl_{JBM} according to Brøchner-Mortensen [3]	Eq. (18)
$(GFR/ECV)_{BM}$	Calculated as GFR_{JBM}/ECV_{BM}	Eq. (19)
Jødal and Brøchner-Mortensen (JBM)		
GFR_{JBM}	Calculated as Cl_{JBM} according to Jødal and Brøchner-Mortensen [6]	Eqs (4) and (5)
ECV_{JBM}	Calculated from V_1 and Cl_1, according to the new method presented here	Eq. (24)
$(GFR/ECV)_{JBM}$	Calculated as GFR_{JBM}/ECV_{JBM}	Eq. (25)

ECV, extracellular fluid volume; GFR, glomerular filtration rate; JBM, Jødal and Brøchner-Mortensen.

Participants investigated

The present study comprised a total of 128 individuals (35 women, 66 men and 27 children), all of whom had a complete plasma concentration curve determined after an intravenous bolus injection of ^{51}Cr-EDTA. None had oedema, and they were chosen from three previously published studies: Study I [3] – 68 nephro-urological patients and 17 normal individuals; Study II [7] – 16 patients with type 1 diabetes investigated during periods of moderate hyperglycaemia; Study III [8] – 27 children aged up to 12 years, all suffering from a nephro-urological disorder.

The data pertaining to the investigated individuals are given in Table 3. In the table, the ratio *GFR/ECV* is expressed in unit %/h; for example, for a person with GFR = 90 ml/min and ECV = 12 l

$$\frac{90\,\text{ml/min}}{12\,000\,\text{ml}} = 0.0075/\text{min} = 0.45/\text{h} = 45\,\%/\text{h}.$$

Procedure

The individuals were confined to bed throughout the examination, which lasted for 4–5 h. ^{51}Cr-EDTA of 2–4 MBq and a known amount of T_{1824} (Evans blue) were injected intravenously through the membrane of an indwelling needle with simultaneous injection of 10 ml of isotonic saline. In Study III, the activity of ^{51}Cr-EDTA was reduced to about 0.1 MBq/kg of body weight. Venous blood samples were drawn through an indwelling needle in the contralateral arm. In Study I, the blood samples were taken at time intervals of 15–30 min for 4–5 h. In Study II and Study III, the blood samples were taken 5, 10, 15, 30, 60, 90, 120, 150, 180, 200, 220 and 240 min after the injection, supplemented with a blood sample at 300 min in children with known severely reduced renal function. All blood samples were counted in a well-type counter to 10 000 counts.

The plasma volume was determined using T_{1824} (Evans blue) (see [9] for details on the method). Activity of ^{51}Cr-EDTA at time zero was calculated as the ratio between the injected amount of tracer and plasma volume.

Calculations

Determination of reference GFR

For determination of reference parameters, the measured plasma concentration curve was resolved into a sum

Table 3 Clinical data of the investigated individuals

	Age (years)	Body surface area (m^2)	$GFR = Cl_{EDTA}$ (ml/min)	*GFR/BSA* (ml/min/1.73 m^2)	*ECV* (1000 ml)	*GFR/ECV* (%/h)
Study I, patients (n=68, M/F=39/29)	49.7 (15–81)	1.80 (1.41–2.29)	76.6 (8.2–139)	73.5 (7.6–127.3)	13.6 (6.9–20.2)	34 (3.1–62)
Study I, normal individuals (n=17, M/F=14/3)	26.6 (18–47)	1.73 (1.59–2.01)	101.3 (74–136)	101.2 (79–134)	13.0 (10.6–16.5)	47 (38–62)
Study II (n=16, M/F=13/3)	29.1 (18–50)	1.69 (1.49–1.87)	123.1 (93–186)	126.1 (100–183)	13.5 (10.2–16.7)	55 (42–81)
Study III (n=27, boys/girls=5/22)	6.4 (0.13–12)	0.85 (0.22–1.57)	44.5 (2.6–88)	85.3 (21–121)	4.6 (0.81–10.0)	56 (16–81)

Values are given as mean (range). In total: n=128 (M/F/children=66/35/27).
BSA, body surface area; Cl_{EDTA}, ^{51}Cr-EDTA plasma clearance; ECV, extracellular fluid volume; F, female; GFR, glomerular filtration rate; M, male.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

of two to four monoexponential functions:

$$C_p(t)=\sum c_i e^{-b_i t}. \quad (1)$$

In line with the notation in Brøchner-Mortensen and colleagues [3,6,9], the numbering of the exponentials was chosen so that $b_1 < b_2 < \cdots$, giving a convenient notation, wherein the slowest, most important exponential has number 1, regardless of the number of exponentials involved in the full plasma concentration curve.

The intercept c_1 and the rate constant b_1 of the slowest exponential were calculated by the least-squares fit to the plasma samples drawn 3–4 h after the injection (3–5 h in cases in which a 5 h sample was taken). The remaining exponentials in the full plasma concentration curve were then resolved with a standard peeling-off technique. For about half of the patients in Study I, a total of two exponentials were used, and for all others three or four exponentials were used.

The total plasma clearance (Cl) was determined as the ratio between the injected amount of tracer (Q_0), and the area under the plasma concentration curve:

$$Cl=\frac{Q_0}{\int_0^\infty C_p(t)\,dt}=\frac{Q_0}{\sum c_i/b_i}. \quad (2)$$

In the present study, these clearance values based on the full ^{51}Cr-EDTA concentration curve were used as references values for the glomerular filtration rate, $GFR_{ref} = Cl_{EDTA}$.

Calculation of GFR from one-pool (slope–intercept) clearance

In clinical practice, the full plasma concentration curve is seldom known. However, if at least two blood samples are taken at time points that are so late that only one exponential is left in the plasma concentration curve, then the slope b_1 and intercept c_1 of this final, slowest exponential can be determined. On the basis of this single exponential, a 'one-pool' clearance (also called a slope–intercept clearance or a final-slope clearance) can be calculated:

$$Cl_1=\frac{Q_0}{c_1/b_1}. \quad (3)$$

As Q_0 is divided by an area that is smaller than the full area, Cl_1 will always be higher than the true Cl and must be corrected.

In the present study, we used the following correction formula [6]:

$$GFR_{JBM}=Cl_{JBM}=\frac{Cl_1}{1+f\times Cl_1}, \quad (4)$$

where

$$f=0.0032BSA^{-1.3}, \quad (5)$$

and BSA is the body surface area in m^2. This correction formula is applicable to individuals of all sizes (from small children to very obese adults) and at all levels of renal function, including the very high renal functions at which quadratic corrections fail [6,10], and has been validated in an independent study [11].

Determination of reference ECV

The distribution volume of ^{51}Cr-EDTA is often taken to be equal to the ECV [3,12]. Therefore, in this study we have used ECV synonymously with distribution volume.

From the general tracer-kinetic theory, the distribution volume is related to clearance as follows:

$$ECV=Cl\times\bar{t}, \quad (6)$$

where $\bar{t}$ is the mean transit time for the tracer:

$$\bar{t}=\frac{\int t\times C_p(t)\,dt}{\int C_p(t)\,dt}=\frac{\sum\frac{c_i}{b_i^2}}{\sum c_i/b_i}=\frac{Cl}{Q_0}\sum\frac{c_i}{b_i^2}. \quad (7)$$

Thus,

$$ECV=\frac{Cl^2}{Q_0}\sum\frac{c_i}{b_i^2}=Q_0\frac{\sum\frac{c_i}{b_i^2}}{(\sum c_i/b_i)^2}. \quad (8)$$

These ECV values calculated from the full curve were used as reference values for the extracellular fluid volume, ECV_{ref}.

Approximate ECV from one-pool (slope–intercept) data

An approximation V_1 to the distribution volume can be calculated using only one exponential (i.e. the slowest exponential):

$$V_1=\frac{Cl_1^2}{Q_0}\frac{c_1}{b_1^2}=\frac{Cl_1}{b_1}=\frac{Q_0}{c_1}. \quad (9)$$

Note also that

$$V_1=Cl_1\times\bar{t}_1, \quad (10)$$

where

$$\bar{t}_1=1/b_1, \quad (11)$$

and is the mean transit time corresponding to the slowest exponential. Accordingly, the resulting time is longer than the mean transit time based on the full curve, that is, $\bar{t}_1 > \bar{t}$, and it follows that $V_1 > ECV$.

The Peters method for GFR/ECV and ECV determination from one-pool data

Peters [4] advocated describing the renal function in terms of GFR divided by ECV, a ratio that can be approximated simply as the rate constant of the slowest exponential:

$$\frac{GFR}{ECV}=\frac{Cl}{ECV}=\frac{1}{\bar{t}}\approx\frac{Cl_1}{V_1}=\frac{1}{\bar{t}_1}=b_1. \quad (12)$$

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

This rate constant has been suggested by Peters *et al.* [13] as a measure of renal function. In the present context, we denote this as a simple estimate of *GFR*/*ECV*:

$$\left(\frac{GFR}{ECV}\right)_{\text{simple}} = b_1. \tag{13}$$

However, as noted above, $\bar{t}_1$ overestimates the true mean transit time $\bar{t}$. Peters *et al.* [5] therefore multiplied the rate constant by 1 plus a proportional term. In our notation:

$$\left(\frac{GFR}{ECV}\right)_{\text{Peters}} = b_1(1+mb_1) = b_1 + mb_1^2, \tag{14}$$

where m is a constant value. In the cited study [5], Peters and colleagues found the value $m = 15.4\,\text{min} = 0.0154\ (\text{ml/min/l})^{-1}$ for EDTA. Bird *et al.* [12] has validated the correction using this value of the constant.

In the present study, Peters' method was evaluated by plotting $GFR_{\text{ref}}/ECV_{\text{ref}}$ as a function of b_1 to confirm whether the points did lie on a well-defined curve of the form described by Eq. (14) and whether the constant $m = 15.4\,\text{min}$ could be confirmed.

ECV can also be of interest in itself. As Peters' formula [Eq. (14)] expresses *ECV* only in combination with *GFR*, we calculated a 'Peters' value for *ECV* with the help of GFR_{JBM}:

$$ECV_{\text{Peters}} = \frac{GFR_{\text{JBM}}}{(GFR/ECV)_{\text{Peters}}}. \tag{15}$$

The Brøchner-Mortensen equation for ECV and GFR/ECV determination from one-pool data

Brøchner-Mortensen [3] calculated *ECV* as follows:

$$ECV_{\text{BM}} = \frac{Cl}{b_1}\left[\left(\frac{Cl}{Cl_1}\right)^2 - \frac{Cl}{Cl_1} + 1\right]. \tag{16}$$

This can also be seen as a correction to the simple one-pool value V_1:

$$ECV_{\text{BM}} = V_1\frac{Cl}{Cl_1}\left[\left(\frac{Cl}{Cl_1}\right)^2 - \frac{Cl}{Cl_1} + 1\right]. \tag{17}$$

Equations (16) and (17) are mathematically equivalent.

In the present study, the form of this correction was evaluated in the following way. On the basis of the reference values for *Cl* (*GFR*) and *ECV*, the ratio V_1/ECV was plotted as a function of the ratio Cl_1/Cl. The former ratio is the factor by which the one-pool volume (V_1) overestimates the true volume (*ECV*), whereas the latter ratio is the similar overestimation factor for clearance. In such a plot, Brøchner-Mortensen's ECV equation gives a well-defined curve: for a given value of $x = Cl_1/Cl$, the value of $y = V_1/ECV_{\text{BM}}$ can be calculated from Eq. (17). The resulting plot compares Brøchner-Mortensen's equation [i.e. Eqs (16) and (17)] with the 'true' relation between V_1/ECV and Cl_1/Cl from the reference data.

Brøchner-Mortensen's equation is meant to be used in cases in which only one-pool data are known. Thus, *Cl* must be determined from Cl_1. In the original study [3], a variant of Brøchner-Mortensen's classical clearance correction [9] was used, and the study included only adults. Here, we used instead Cl_{JBM}, that is, the improved correction defined by Eqs (4) and (5), and included both adults and children.

$$ECV_{\text{BM}} = \frac{Cl_{\text{JBM}}}{b_1}\left[\left(\frac{Cl_{\text{JBM}}}{Cl_1}\right)^2 - \frac{Cl_{\text{JBM}}}{Cl_1} + 1\right]. \tag{18}$$

We also calculated a corresponding 'Brøchner-Mortensen' estimate of *GFR*/*ECV*:

$$\left(\frac{GFR}{ECV}\right)_{\text{BM}} = \frac{GFR_{\text{JBM}}}{ECV_{\text{BM}}}. \tag{19}$$

A new, simple method for ECV and GFR/ECV determination from one-pool data

The present study revealed the following simple relation:

$$\frac{V_1}{ECV} = 2\frac{Cl_1}{Cl} - 1. \tag{20}$$

This new relation can also be expressed as follows:

$$\left(\frac{V_1}{ECV} - 1\right) = 2\times\left(\frac{Cl_1}{Cl} - 1\right), \tag{21}$$

that is, V_1 overestimates *ECV* twice as much as Cl_1 overestimates *Cl* (e.g. if Cl_1 is 20% higher than *Cl*, then V_1 is 40% higher than *ECV*). A theoretical basis for this finding is given in Appendix 1, along with an analysis of its degree of accuracy.

Using Cl_{JBM} from Eq. (4), we can calculate the relative overestimation of clearance by Cl_1 as follows:

$$\frac{Cl_1}{Cl_{\text{JBM}}} - 1 = f\times Cl_1, \tag{22}$$

leading to a new formula for the relative overestimation of *ECV* and thereby a new formula for calculation of *ECV* from V_1:

$$\frac{V_1}{ECV_{\text{JBM}}} - 1 = 2f\times Cl_1, \tag{23}$$

$$ECV_{\text{JBM}} = \frac{V_1}{1+2f\times Cl_1}, \tag{24}$$

where f is the factor given by Eq. (5).

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

The ratio GFR/ECV was estimated for this new method as well:

$$\left(\frac{GFR}{ECV}\right)_{\mathrm{JBM}} = \frac{GFR_{\mathrm{JBM}}}{ECV_{\mathrm{JBM}}}. \qquad (25)$$

Statistics and agreement analysis

A significance level of 0.05 was used, and all reported P values are two sided.

For each method, values of estimated ECV (ECV_{est}) were calculated and compared with reference ECV (ECV_{ref}) values. The differences between ECV_{est} and ECV_{ref} were tested for normal distribution using the Shapiro–Wilk W-test, and the F-test was used to compare variances between children and adults. Bias and SD of differences were calculated, and 95% limits of agreement (LOA) were calculated as bias ± 1.96SD. The Student paired t-test (estimate versus reference) was used to test for bias being significantly different from zero. Relative differences were shown graphically as Bland–Altman plots [14,15]; that is, relative differences were plotted as a function of $(ECV_{\mathrm{est}} + ECV_{\mathrm{ref}})/2$. Similarly for data on *GFR/ECV*.

To see whether all methods had similar variance (SD^2) or not, Levene's test was used. In case of significant difference, the methods were compared pairwise by the F-test. As we were comparing four methods [simple, Peters, Brøchner-Mortensen (BM) and Jødal and Brøchner-Mortensen (JBM)], there were six pairwise comparisons; hence, to maintain an overall significance level of 0.05, only P values less than 0.0085 were considered statistically significant in the pairwise comparisons: $(1–0.0085)^6 = 0.95$.

The software StatsDirect, version 2.7.8 (*http://www.statsdirect.com*) was used for the statistical calculations.

Results

Test of equations using reference values

Figure 1 shows the comparison of Peters' relation [Eq. (14)], with 'true' values (i.e. reference values). On average, the data do follow a curve of the form $b_1 + mb_1^2$, but our data point to a factor $m \approx 20$ min rather than $m = 15.4$ min.

Figure 2 shows a comparison between Brøchner-Mortensen's ECV equation [Eq. (16)] and 'true' values, showing a systematic underestimation of V_1/ECV at the highest levels.

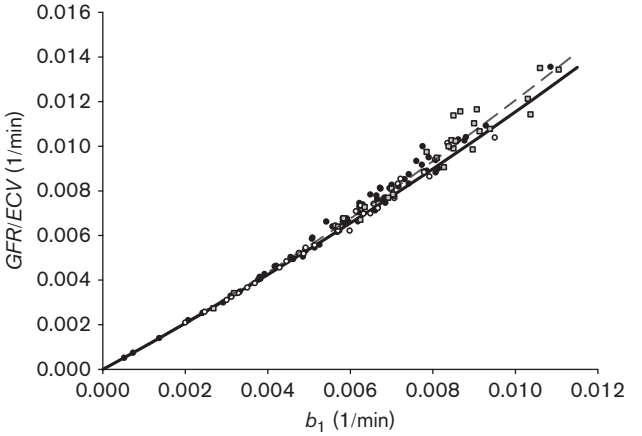
Also in Fig. 2 is plotted the line $y = 2x - 1$, which is the basis for the new ECV_{JBM} calculation [see Eqs (20)–(24) in the Calculations section].

Comparison of methods when applied to one-pool values

Four methods were compared with reference values for ECV and GFR/ECV:

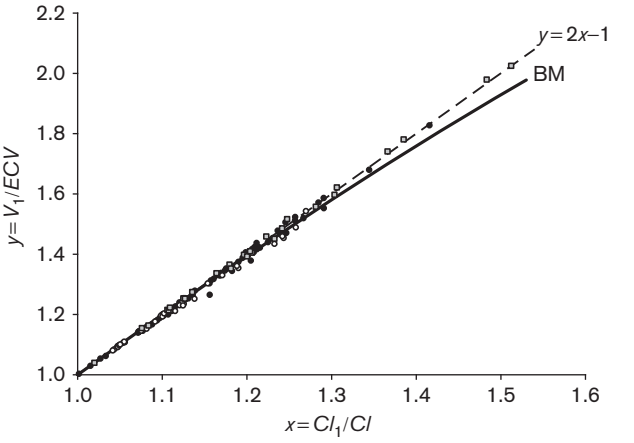
(1) the simple (slope-only) method for GFR/ECV and the simple V_1 estimate for ECV [see Eqs (13) and (9), respectively];
(2) Peters' method [see Eqs (14) and (15)];
(3) Brøchner-Mortensen's method [BM, see Eqs (18) and (19)];
(4) the new method [JBM, see Eqs (24) and (25)].

Fig. 1



Test of Peters' relation on reference data: reference *GFR/ECV* as a function of b_1, both in units of 1/min. Women: open circles. Men: full circles. Children: grey squares. Full line is $(GFR/ECV)_{\mathrm{Peters}} = b_1 + 15.4b_1^2$. Dashed line is fit to data: $GFR/ECV = 1.007b_1 + 19.9b_1^2$. ECV, extracellular fluid volume; GFR, glomerular filtration rate.

Fig. 2



Test of Brøchner-Mortensen's (BM) relation on reference data: ratio $y = V_1/ECV$ as a function of ratio $x = Cl_1/Cl$, both from reference data. Women: open circles. Men: full circles. Children: grey squares. Full line calculated from ECV_{BM} [Eq. (17)]. Dashed line is $y = 2x - 1$. ECV, extracellular fluid volume.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

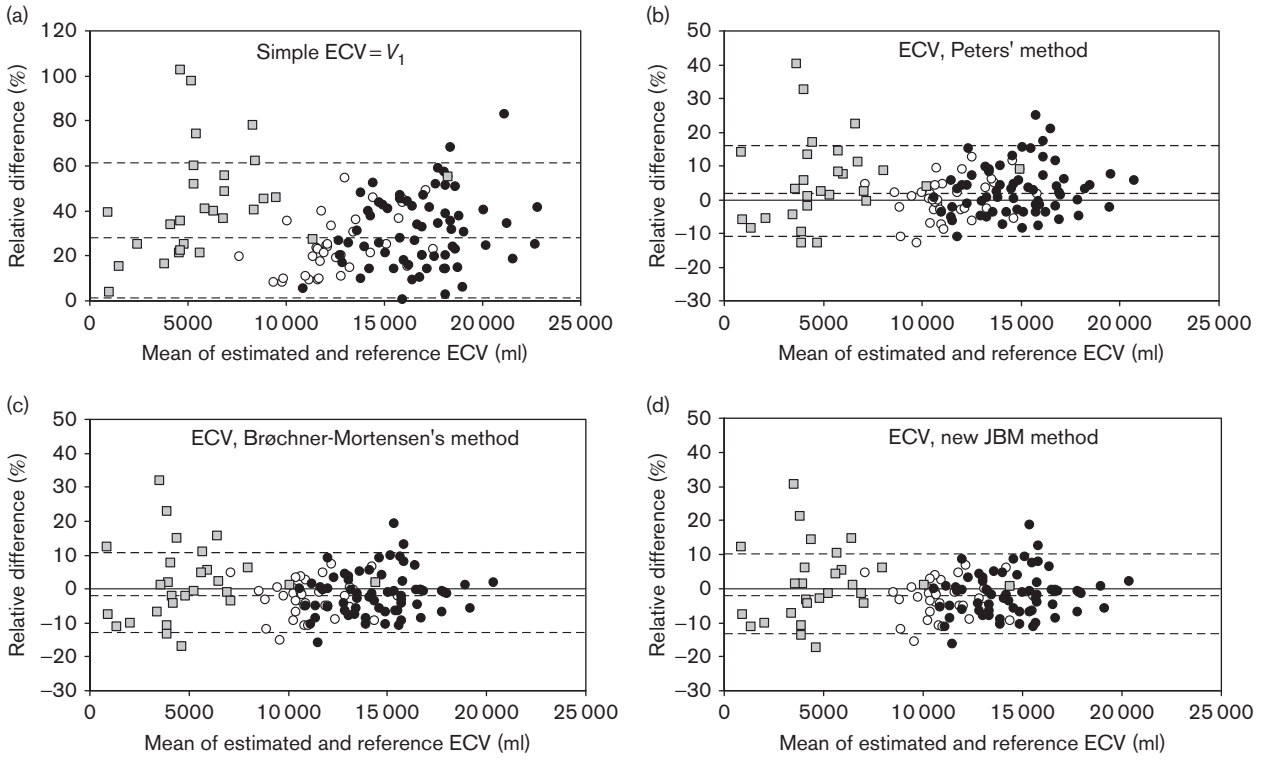
ECV

The differences between estimated and reference ECV values were found to be normally distributed for each method if adults and children were considered separately and data were logarithmized. The *F*-test for each method showed SD^2 to differ between adults and children ($P = 0.03$ for the simple V_1 method; $P \leq 0.0001$ for the other three methods). Bias, SD and LOA were calculated on logarithmic values but are reported as percentage deviations from reference values – for example, $\text{bias}\% = (\exp(\text{bias}_{\ln}) - 1) \times 100\%$.

Figure 3 shows Bland–Altman plots for relative differences between estimated and reference *ECV*. The corresponding results for bias and SD are given in Table 4. For adults, all methods had statistically significant bias ($P < 0.03$), whereas for children bias was only significant for the simple V_1 method.

Levene's test showed a significant difference between variances for adults ($P < 0.0001$) but not for children ($P = 0.2$). For adults, the pairwise comparisons showed SD^2 to be significantly higher for the simple V_1 method

Fig. 3



Results for estimation of ECV from one-pool data according to the investigated methods: (a) simple V_1 estimate; (b) Peters' method; (c) Brøchner-Mortensen's method (BM); (d) the new method (JBM). On the vertical axis is given the relative difference between the estimated and reference ECV values. Bias and 95% limits of agreement for adults are shown as dashed lines (for children, see Table 4). Women: open circles. Men: full circles. Children: grey squares. Note that the simple method has other vertical scales than the other methods. ECV, extracellular fluid volume; JBM, Jødal and Brøchner-Mortensen.

Table 4 Comparison between estimated ECV and reference ECV

	Adults			Children		
Method	Bias (%)	SD (%)	95% LOA	Bias (%)	SD (%)	95% LOA
Simple (one-pool V_1)	+28	12.6	+1.3 to +61	41	17.5	+3.0 to +94
Peters	+1.5	7.0	−11.1 to +15.9	+4.7	12.4	−16.7 to +32
Brøchner-Mortensen (BM)	−2.0	6.3	−13.1 to +10.5	+1.2	11.4	−18.1 to +25
New JBM	−2.4	6.3	−13.4 to +10.1	+0.7	11.2	−18.3 to +24

All values have been determined from logarithmic differences from the reference values and then converted to a percentage of the reference value.
For adults, all biases were significantly different from zero, but only the simple V_1 had SD^2 differing significantly from those of other methods. For all methods, SD^2 was significantly higher for children than for adults.
JBM, Jødal and Brøchner-Mortensen; LOA, limits of agreement.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

than for any other method ($P < 0.0001$), whereas SD^2 did not differ between any of the other three methods ($P > 0.3$ in these comparisons).

GFR/ECV

Likewise, for GFR/ECV it was found that separation of adults and children and logarithmization of data gave the best results in the normality test; for the logarithmized data, only Peters' method showed signs of non-normality (adults only, $P = 0.04$). We proceeded using the *F*-test, bearing in mind that the conclusions should be tentative in the case of Peters' method for GFR/ECV. For all methods, except the simple b_1 method, variance (SD^2) was significantly higher for children than for adults ($P = 0.07$ for the simple method; $P \leq 0.004$ for the other three methods).

Figure 4 and Table 5 show results for *GFR/ECV* for the four methods. For adults, bias was statistically significant for all methods ($P \leq 0.0003$), whereas in children this was the case only for the simple method and Peters' method ($P \leq 0.0006$ for these methods, $P > 0.3$ for BM and JBM).

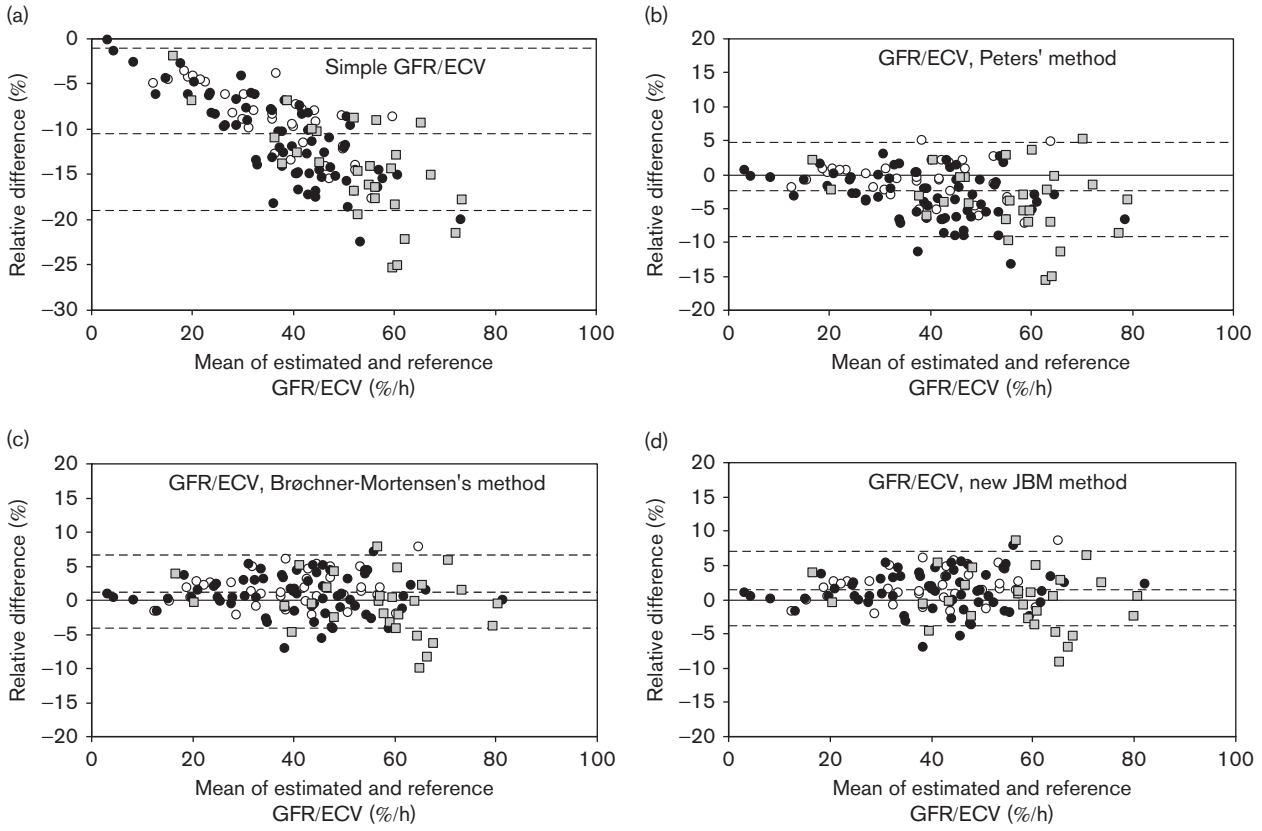
Levene's test showed SD^2 to differ significantly between methods for adults ($P < 0.0001$) but not for children ($P = 0.2$). The pairwise *F*-tests for adults showed the simple b_1 rate constant to have larger SD^2 compared with Peters' method ($P < 0.001$), which had higher SD^2 than both the BM and JBM methods ($P \leq 0.004$). Variance of BM and JBM did not differ significantly ($P = 0.9$).

Discussion

The clinical role of ECV and GFR/ECV

The kidneys play a key role in the regulation of body fluids and elimination of waste products. Renal function can be expressed as GFR in ml/min, a number that is often scaled to BSA, expressing GFR in ml/min/1.73 m^2. This scaling has been empirically proven to be useful because standard materials show normal values of GFR in ml/min/1.73 m^2 to be relatively independent of age from children above the age of 2 [16] up to adults aged 40–50 years, after which it decreases at a rate of

Fig. 4



Results for estimation of *GFR/ECV* from one-pool data according to the investigated methods: (a) simple (slope-only) method; (b) Peters' method; (c) Brøchner-Mortensen's method (BM); (d) the new method (JBM). On the vertical axis is given the relative difference between estimated and reference GFR/ECV values. Bias and estimated 95% limits of agreement for adults are shown as dashed lines (for children, see Table 5). Women: open circles. Men: full circles. Children: grey squares. Note that the simple method has other vertical scales than the other methods. ECV, extracellular fluid volume; GFR, glomerular filtration rate; JBM, Jødal and Brøchner-Mortensen.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Table 5 Comparison between estimated GFR/ECV and reference GFR/ECV

	Adults			Children		
Method	Bias (%)	SD (%)	95% LOA	Bias (%)	SD (%)	95% LOA
Simple (rate constant b_1)	−10.5	5.3	−19.1 to −1.1	−14.4	6.9	−24.9 to −2.6
Peters	−2.5	3.7	−9.2 to +4.7	−4.0	5.7	−13.9 to +6.9
Brøchner-Mortensen (BM)	+1.0	2.8	−4.2 to +6.6	−0.7	4.5	−9.0 to +8.2
New JBM	+1.4	2.7	−3.9 to +6.9	−0.2	4.4	−8.2 to +8.6

All values have been determined from logarithmic differences from the reference values and then converted to a percentage of the reference value.
For adults, all methods had bias significantly different from zero, and SD^2 was significantly different in this order: simple > Peters > BM = JBM. Children had significantly higher SD^2 than adults for all methods except the simple rate constant b_1.
JBM, Jødal and Brøchner-Mortensen; LOA, limits of agreement.

9–10 ml/min/1.73 m^2 per decade [17,18]. However, apart from the general expectation that a larger body needs larger filtering, there is no physiological reason why renal function should be coupled with BSA. Furthermore, the result will depend on the choice and accuracy of the formula used for calculation of BSA [2].

In contrast, scaling GFR to ECV has a direct physiological interpretation: it links the regulation rate (GFR) to the amount of fluid to be regulated (ECV). Expressing GFR/ECV in the unit %/h can help give an intuitive understanding of the number. For example, 45%/h (as in the earlier example) means that the volume of plasma filtered by the kidneys in 1 h corresponds to 45% of the total volume involved (ECV). A useful unit conversion is 1 ml/min/l = 6%/h. It is of course also possible to express GFR/ECV as scaled to a 'standard ECV'. If, for instance, 12 l is used as 'standard ECV', we find 50%/h = 100 ml/min/12 l.

We have studied simplified methods for the determination of GFR/ECV after an intravenous bolus injection of ^{51}Cr-EDTA on the basis of two different approaches: either from the rate constant b_1 of the final exponential (the simple method and Peters' method) or from the ratio between one-pool clearance and total plasma clearance (Brøchner-Mortensen's method and the new JBM method).

Correctness of methods based on b_1 (simple and Peters)

As described previously, the ratio $GFR/ECV = Cl/ECV$ can be calculated using the rate constant b_1 as a first approximation, followed by a correction, again based only on b_1. The underlying assumption in such a method is that GFR/ECV is a well-defined function of b_1.

As seen by the 'true' values in Fig. 1, the ratio GFR/ECV can be reasonably well described as a function of b_1 ($GFR/ECV = b_1 + mb_1^2$), although GFR/ECV is slightly underestimated at high levels of b_1 (high renal function) when the original value of $m = 15.4$ min is used (Peters *et al.* [5] and Bird *et al.* [12]). The current data point to a more reliable value of $m \approx 20$ min.

This altered value of the constant is in fact in line with a study by Bird *et al.* [19]. Table I of that study gives for ^{51}Cr-EDTA a correction that, in our notation, is $0.965b_1 + 0.00204b_1^2$ (ml/min/l)$^{-1} = 0.965b_1 + 20.4b_1$ (min). However, in the later study [12], the original version of the correction was used: that is, $m = 15.4$ min $= 0.0154$ (ml/min/l)$^{-1}$.

Correctness of methods based on Cl_1/Cl (BM and JBM)

This principle is based on the relationship between V_1/ECV and Cl_1/Cl. Using the BM method for determination of ECV [3] [i.e. Eq. (16) or (17)] on the basis of reference values, it was shown that there is indeed a well-defined relationship between $y = V_1/ECV$ and $x = Cl_1/Cl$ (Fig. 2), although with the BM method V_1/ECV is underestimated to some extent at high renal function leading to a minor overestimation of ECV and a corresponding underestimation of GFR/ECV.

As an unexpectedly simple result, we found the data in Fig. 2 to be very close to the line $y = 2x - 1$ (dashed line). In retrospect, such a relation might have been expected from the study by Peters *et al.* [5]. In an appendix, they argued that the gradient of an equation for V_1/ECV would be approximately twice the gradient of an equation for Cl_1/Cl. However, the authors did not analyse this result further, nor did they evaluate the extent to which it would be correct for different values of renal function. In Appendix 1 of the present study, the relationship has been analysed, and it has been argued that the relationship can be expected to be fairly accurate for all levels of renal function. The equation for the resulting ECV estimate, ECV_{JBM}, is given in Eq. (24).

Comparison of the methods for clinical use

All the methods have been developed for use in clinical situations in which only the injected amount of activity and one-pool data (i.e. the final exponential of the plasma concentration curve) are known.

Results for ECV (Fig. 3 and Table 4) show that the simple V_1 method yields poor results with respect to both bias and spread. Although the other three methods have a statistically significant bias, a small percentage of bias is not important from a clinical point of view and will drown in the spread for the individual patient (as indicated by the 95% LOA). The larger spread for children than for adults can be explained by the findings in Jødal and

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Brøchner-Mortensen [6] that the area under the fast exponential(s) has a larger variation in children than in adults.

For *GFR/ECV* (Fig. 4 and Table 5), the simple rate constant method might be used if one is aware that especially high values (Fig. 4) will have bias compared with the 'true' GFR/ECV and that the method has a rather large spread. The dependence on renal function means that any single number for 'bias' of the simple rate constant method will depend on the population, and the number in Table 5 for this method should be considered only as an example.

With regard to the spread of *GFR/ECV*, the BM and JBM methods had especially favourable results. Rounding the numbers, the 95% LOA were from −4% to +7% for adults and ±9% for children. These are very narrow limits for clinical data and at least as good as the corresponding LOA for clearance (and thereby GFR) found in Jødal and Brøchner-Mortensen [6]. Largely on the basis of the same data as in the present study, Jødal and Brøchner-Mortensen found that 95% LOA for clearance were (in round numbers) ±7% for adults and from −11% to +13% for children. The narrow LOA for GFR_{JBM}/ECV_{JBM} is because of the variable f being present in both the numerator and the denominator, giving the effect that estimation errors in f to some extent cancel out.

JBM versus *BM:* As seen by the curves in Fig. 2, the JBM and BM methods will in most, but not all, cases give very similar results. The value on the horizontal axis is $x = Cl_1/Cl = 1 + f \times Cl_1$, using the JBM formulation [Eq. (4)]; hence, the differences are seen when either f or Cl_1 or both have high values. The factor f is high for small subjects [Eq. (5)], whereas one-pool clearance Cl_1 is high when renal function is high. Thus, the new JBM method for calculation of *ECV* and *GFR/ECV* will be more accurate than the BM method in individuals with high renal function, especially in children. As the present study included only a few patients with these characteristics, this higher accuracy was not reflected in the overall bias and SD but is clearly seen in the rightmost part of Fig. 2.

GFR/ECV versus GFR scaled to 1.73 m^2

Unlike the usual scaling of GFR to BSA of 1.73 m^2, the ratio GFR/ECV has a physiological interpretation. As mentioned in the introduction, GFR scaled to 1.73 m^2 does not always reflect renal function, which can be seen as a result of the lack of a physiological link between GFR and BSA. For instance, if an administered drug has a distribution volume approximately corresponding to ECV and is excreted by the kidneys, then GFR/ECV will be a more direct measure than GFR/BSA for determining how fast the drug is cleared from the body.

However, in cases of hypohydration or hyperhydration, it can be expected that ECV is changed without a similar change in GFR. Besides, scaling of GFR to 1.73 m^2 has long historical roots [1] and well-established normal ranges [16–18]. For these reasons, GFR/ECV will likely not replace GFR scaled to 1.73 m^2, but can give useful supplementary information.

Normal ranges for GFR/ECV will need to be established. The recent study by Peters *et al.* [20] on a large group (1878 individuals) of healthy potential renal transplant donors seems a good basis on which to determine normal ranges for adults. They found mean values of GFR/ECV to be 7.15 ± 1.23 ml/min/l for men and 7.40 ± 1.39 ml/min/l for women, corresponding to 42.9 ± 7.4 and 44.4 ± 8.3%/h, respectively. With regard to age dependency, the researchers found the following regression lines for individuals above 40 years of age:

$$(9.5 - 0.051 \times \text{age})\,\text{ml/min/l} = (57 - 0.31 \times \text{age})\,\%/\text{h} \quad \text{for men above 40 years of age,}$$

$$(11.3 - 0.079 \times \text{age})\,\text{ml/min/l} = (67.8 - 0.47 \times \text{age})\,\%/\text{h} \quad \text{for women above 40 years of age.}$$

In individuals younger than 30 years, the mean values ± SD were 7.63 ± 0.91 ml/min/l = 45.8 ± 5.5%/h for men and 8.26 ± 1.41 ml/min/l = 49.6 ± 8.5%/h for women. Although the researchers found the differences between men and women to be statistically significant, Fig. 1B of the paper indicated a large overlap between the data for men and women (as will mean ± 2 SD from the above data).

GFR assessed as ^{51}Cr-EDTA clearance or as inulin clearance

It is generally accepted that the renal clearance of inulin is the most accurate measure of GFR. However, the British Nuclear Medicine Society (BNMS) guidelines on GFR measurements from plasma samples recommend 'that the plasma clearance of EDTA from venous samples be taken as the standard measure of GFR' [21]. Inulin and ^{51}Cr-EDTA differ slightly in their kinetics: renal clearance of inulin is about 10% higher than renal clearance of EDTA, but EDTA has a small extrarenal clearance of about 3.7 ml/min for adults and about 3.6 ml/min/1.73 m^2 for children [22], corresponding to the following conversion:

$$Cl_{\text{inulin}} = 1.1 \times (Cl_{\text{EDTA}} - Cl_{\text{er}}), \qquad (26)$$

with

$$Cl_{\text{er}} = 3.7\ \text{ml/min for adults,}$$

$$Cl_{\text{er}} = 3.6\ \text{ml/min/1.73 m}^2\ \text{for children.}$$

Using Cl_{er} = 3.7 ml/min, we find that $Cl_{\text{inulin}} < Cl_{\text{EDTA}}$ (by up to about 4 ml/min) for Cl_{EDTA} is less than 40 ml/min, whereas Cl_{inulin} is between 5 and 10% higher than Cl_{EDTA} for Cl_{EDTA} greater than 82 ml/min.

In the present study, we have taken EDTA clearance as the measure of GFR. If inulin clearance is taken as the measure of GFR, then [according to Eq. (26)] the GFR/ECV values will be slightly lower for very low renal function, about the

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

same for moderately reduced renal function, and 5–10% higher for normal and high renal function. However, when measured in percentage, as here, the spread and bias are virtually unaffected (data not shown).

Conclusion

GFR/ECV is a physiologically relevant measure of renal function and can be determined with good precision. We found the best precision using GFR determined by the method of Jødal and Brøchner-Mortensen [Eqs (4) and (5)] combined with ECV determined either with Brøchner-Mortensen's method or with the new JBM method [Eq. (24)].

The formula for GFR/ECV presented by Peters [5] [Eq. (14)] gives reasonably precise values, but with the factor 15.4 min the method underestimates GFR/ECV at high renal function, according to our data.

The new JBM method for determination of ECV was theoretically more correct than the BM method. In practice, the difference is very small for most patients but will be seen in patients (especially children) with high renal function.

Expressing renal function as the ratio GFR/ECV gives a physiologically relevant measure of the renal function, which can be determined with at least as high precision as when using GFR. This can be an important supplement to the usual scaling of GFR to BSA of 1.73 m^2, but normal ranges will need to be established.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Heaf JG. The origin of the 1.73-m^2 body surface area normalization: problems and implications. *Clin Physiol Funct Imaging* 2007; **27**: 135–137.
2 Geddes CC, Woo YM, Brady S. Glomerular filtration rate – what is the rationale and justification of normalizing GFR for body surface area? *Nephrol Dial Transplant* 2008; **23**:4–6.
3 Brøchner-Mortensen J. A simple single injection method for determination of the extracellular fluid volume. *Scand J Clin Lab Invest* 1980; **40**: 567–573.
4 Peters AM. Expressing glomerular filtration rate in terms of extracellular fluid volume. *Nephrol Dial Transplant* 1992; **7**:205–210.
5 Peters AM, Henderson BL, Lui D, Blunkett M, Cosgriff PS, Myers MJ. Appropriate corrections to glomerular filtration rate and volume of distribution based on the bolus injection and single-compartment technique. *Physiol Meas* 1999; **20**:313–327.
6 Jødal L, Brøchner-Mortensen J. Reassessment of a classical single injection ^{51}Cr-EDTA clearance method for determination of renal function in children and adults. Part I: analytically correct relationship between total and one-pool clearance. *Scand J Clin Lab Invest* 2009; **69**: 305–313.
7 Brøchner-Mortensen J. Glomerular filtration rate and extracellular fluid volumes during normoglycemia and moderate hyperglycemia in diabetics. *Scand J Clin Lab Invest* 1973; **32**:311–316.
8 Brøchner-Mortensen J, Haahr J, Christoffersen J. A simple method for accurate assessment of the glomerular filtration rate in children. *Scand J Clin Lab Invest* 1974; **33**:139–143.
9 Brøchner-Mortensen J. A simple method for the determination of glomerular filtration rate. *Scand J Clin Lab Invest* 1972; **30**:271–274.
10 Brøchner-Mortensen J, Jødal L. Reassessment of a classical single injection ^{51}Cr-EDTA clearance method for determination of renal function in children and adults. Part II: empirically determined relationships between total and one-pool clearance. *Scand J Clin Lab Invest* 2009; **69**:314–322.
11 Peters AM. Re-evaluation of the new Jødal–Brøchner-Mortensen equation for one-pool correction of slope–intercept measurement of glomerular filtration rate. *Nucl Med Commun* 2011; **32**:375–380.
12 Bird NJ, Michell AR, Peters AM. Accurate measurement of extracellular fluid volume from the slope/intercept technique after bolus injection of a filtration marker. *Physiol Meas* 2009; **30**:1371–1379.
13 Peters AM, Henderson BL, Lui D. Comparison between terminal slope rate constant and 'slope/intercept' as measures of glomerular filtration rate using the single-compartment simplification. *Eur J Nucl Med* 2001; **28**:320–326.
14 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**:307–310.
15 Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995; **346**: 1085–1087.
16 Piepsz A, Tondeur M, Ham H. Revisiting normal ^{51}Cr-ethylenediaminetetraacetic acid clearance values in children. *Eur J Nucl Med Mol Imaging* 2006; **33**: 1477–1482.
17 Granerus G, Aurell M. Reference values for ^{51}Cr-EDTA clearance as a measure of glomerular filtration rate. *Scand J Clin Lab Invest* 1981; **41**:611–616.
18 Grewal GS, Blake GM. Reference data for ^{51}Cr-EDTA measurements of the glomerular filtration rate derived from live kidney donors. *Nucl Med Commun* 2005; **26**:61–65.
19 Bird NJ, Peters C, Michell AR, Peters AM. Suitability of a simplified technique based on iohexol for decentralized measurement of glomerular filtration rate. *Scand J Urol Nephrol* 2008; **42**:472–480.
20 Peters AM, Perry L, Hooker CA, Howard B, Neilly MDJ, Seshadri N, *et al.* Extracellular fluid volume and glomerular filtration rate in 1878 healthy potential renal transplant donors: effect of age, gender, obesity and scaling. *Nephrol Dial Transplant* 2012; **27**:1429–1437.
21 Fleming JS, Zivanovic MA, Blake GM, Burniston M, Cosgriff PS. Guidelines for the measurement of glomerular filtration rate using plasma sampling. *Nucl Med Commun* 2004; **25**:759–769.
22 Brøchner-Mortensen J. Current status on assessment and measurement of glomerular filtration rate. *Clin Physiol* 1985; **5**:1–17.

Appendix 1

It has been empirically found that V_1 overestimates ECV twice as much as Cl_1 overestimates Cl [Fig. 2 and Eqs (20) and (21)]. The aim of this appendix is to give a theoretical basis to this finding and analyse its accuracy.

Using Eqs (2) and (3), the ratio Cl_1/Cl can be written as follows:

$$\frac{Cl_1}{Cl}=\frac{b_1}{c_1}\sum\frac{c_i}{b_i}=1+\frac{b_1}{c_1}\sum_{i\geq 2}\frac{c_i}{b_i}\equiv 1+\eta, \qquad (A1)$$

where η represents the correction [i.e. in the notation of Eq. (4), $\eta = f \times Cl_1$, not assuming f to be given by Eq. (5)]. Using the leftmost equal sign in both Eqs (8) and (9), we similarly find

$$\frac{V_1}{ECV}=\frac{\frac{Cl_1^2}{Q_0}\frac{c_1}{b_1^2}}{\frac{Cl^2}{Q_0}\sum\frac{c_i}{b_i^2}}=\frac{\left(\frac{Cl_1}{Cl}\right)^2}{1+\frac{b_1^2}{c_1}\sum_{i\geq 2}\frac{c_i}{b_i^2}}=\frac{(1+\eta)^2}{1+\beta}, \qquad (A2)$$

where β is the summed term of the denominator. Therefore,

$$\frac{V_1}{ECV}=\frac{1+2\eta+\eta^2}{1+\beta}, \qquad (A3)$$

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

where

$$\eta \equiv \frac{b_1}{c_1}\sum_{i\geq 2}\frac{c_i}{b_i} \approx \frac{c_2}{c_1}\frac{b_1}{b_2}, \qquad (A4)$$

$$\beta \equiv \frac{b_1^2}{c_1}\sum_{i\geq 2}\frac{c_i}{b_i^2} \approx \frac{c_2}{c_1}\frac{b_1^2}{b_2^2}. \qquad (A5)$$

The approximate values for η and β correspond to using no more than two exponentials in the plasma concentration curve, ignoring exponentials faster than those. The approximations are used here only to see that η^2 and β are both second-order values in terms of b_1/b_2, a ratio that by definition is less than 1. Thus, expressing Eq. (A3) to first order we find:

$$\frac{V_1}{ECV} \approx 1+2\eta=1+2\left(\frac{Cl_1}{Cl}-1\right), \qquad (A6)$$

in accordance with the empirical relation given by Eq. (20).

It is worth considering the accuracy of Eq. (A6) compared with the exact Eq. (A3). As the ignored terms η^2 and β are coupled with the corrections for Cl_1 and V_1, the approximation will be good when the corrections are small, which will be the case for low renal function. But what happens for high renal function? In the present study, both η^2 and β had a median value of 0.02 but maximum values of 0.13 and 0.26, respectively.

First, it should be noted that both the numerator and denominator of Eq. (A3) have a positive term ignored by the approximation in Eq. (A6). Therefore, the errors above and below the fraction line will to some extent cancel each other, giving a result that is more accurate than the individual approximations. Second, the ignored terms are correlated, as seen by Eqs (A4) and (A5): $\eta^2 \approx (c_2/c_1)\beta$. This means that the approximation errors in the numerator and denominator will grow together, thereby keeping a relatively high accuracy for Eq. (A6) even when corrections are large.

In conclusion, the empirical relation in Eq. (20) has been given a theoretical explanation, and the relation is expected to be relatively accurate for all levels of renal function. This expectation is in line with the data shown in Fig. 2.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.