

Validation of Tikhonov adaptively regularized gamma variate fitting with 24-h plasma clearance in cirrhotic patients with ascites

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Abstract

Purpose The aim was to compare late-time extrapolation of plasma clearance (CL) from Tikhonov adaptively regularized gamma variate fitting (Tk-GV) and from mono-exponential (E1) fitting.

Methods Ten ^{51}Cr -ethylenediaminetetraacetic acid bolus IV studies in adults—8 with ascites—assessed for liver transplantation, with 12–16 plasma samples drawn from 5-min to 24-h, were fit with Tk-GV and E1 models and CL results were compared using Passing-Bablok fitting.

Results The 24-h $CL(\text{Tk-GV})$ values ranged from 11.4 to 79.7 ml/min. Linear regression of 4- versus 24-h $CL(\text{Tk-GV})$ yielded no significant departure from a slope of 1, whereas the 4- versus 24-h $CL(\text{E1})$ slope, 1.56, was significantly increased. For $CL(\text{Tk-GV-24-h})$ versus $CL(\text{E1-24-h})$, there was a biased slope and intercept (0.85, 5.97 ml/min). Moreover, the quality of fitting of 24-h data was significantly better for Tk-GV than for E1, as follows. For 10 logarithm of concentration curves, higher r values were obtained for each Tk-GV fit (median 0.998) than for its corresponding E1 fit (median 0.965), with $p < 0.0001$ (paired t -test of z -statistics from Fisher r - z transformations). The E1 fit quality degraded with increasing V/W [volume of distribution (l) per kg body weight, $p = 0.003$]. However, Tk-GV fit quality versus V/W was uncorrelated ($p = 0.8$).

Conclusion $CL(\text{E1})$ values were dependent on sample time and the quality of fit was poor and degraded with increasing ascites, consistent with current opinion that $CL(\text{E1})$ is contraindicated in ascitic patients. $CL(\text{Tk-GV})$ was relatively more accurate and the good quality of fit was unaffected by ascites. $CL(\text{Tk-GV})$ was the preferred method for the accurate calculation of CL and was useful despite liver failure and ascites.

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Abbreviations

α	Shape parameter of GV, volume scale factor, and plasma leak term Eq. 1
AUC	Area under the curve of concentration in time, i.e. $\int_0^\infty C_{obs}(t) dt$
β	Rate constant for GV (per min)
CL	Plasma clearance, D/AUC

$C_{obs}(t)$	Observed concentration, in Bq/ml, often scaled as dose percent per ml
$C(t)$	Continuous function for estimation of $C_{obs}(t)$
CV	Coefficient of variation, SD/mean
D	The dosage in Bq or per cent
E1, E2,	Sum of exponential terms model with 1, 2 or n
En	terms, also, 1, 2, or n -compartment model
GV	Gamma variate, $C(t) = Kt^{\alpha-1}e^{-\beta t}$
Γ	Tikhonov matrix [do not confuse with $\Gamma(x)$]
$\Gamma(x)$	The gamma function, $\Gamma(x) = \int_0^{\infty} t^{x-1}e^{-t}dt$, is a generalization of the factorial, $\Gamma(n) = (n-1)!$
K	Constant of proportionality for GV
MRT	Mean residence time
$\Psi(\alpha)$	(Psi) digamma function of α is $\Psi(\alpha) = d[\ln \Gamma(\alpha)]/d\alpha = \Gamma'(\alpha)/\Gamma(\alpha)$
$s_{\alpha}, s_{\beta}, s_{CL}$	SDs of α, β, CL for Tk-GV Eq. 4
s_V	SD of V from Tk-GV Eq. 5
SD	Standard deviation
Tk-GV	Tikhonov regularization of GV fit of CV of CL
V	Volume of distribution of marker (ml or l)

Introduction

This paper presents a validation of a curve fit method for measuring plasma clearance (CL , ml/min). The CL method validated here uses Tikhonov adaptively regularized gamma variate (Tk-GV) fitting. The word “adaptive” is used to imply minimization of relative error of CL as opposed to an ordinary curve fit minimization (Appendix and [13]). In general, radiometric CL is indicated:

- To detect nephrotoxicity of chemotherapy drugs, especially in children
- For many chemotherapeutic agents’ dose calculations
- For detection of renal failure in patients in whom (a) serum creatinine results might be misleading, (b) missing a decline in renal function might be disastrous e.g. single kidney, renovascular disease or renal transplant and (c) a 24-h clearance measurement is difficult, e.g., in the elderly or those with learning difficulties
- For assessment of potential live donors for kidney transplantation
- For the evaluation and follow-up of chronic renal disease
- For the evaluation of single kidney function in conjunction with relative renal function measurements from static or dynamic radionuclide imaging [2]

However, utilization of radiometric CL methods is spotty with only occasional centres performing them, with needed but inconsistently applied CL -value correction factors, without quality control of the individual CL -values, and in

certain cases, current methods are relatively contraindicated. For example, liver failure, ascites, oedema and low CL are identified in the 2004 British Nuclear Medicine Society Guidelines as having problematically inaccurate CL -values [2]. On the other hand, Xirouchakis et al. [14] outline the clinical importance of CL measurements in liver failure: “Renal dysfunction is a well-established predictor of mortality in both acute liver failure and cirrhosis, particularly following the development of complications, such as sepsis. Inulin clearance and other direct methods using injected exogenous radiolabelled substances [^{51}Cr -EDTA (ethylenediamine tetra-acetic acid), ^{125}I -Iothalamate, $^{99\text{m}}\text{Tc}$ -DTPA (diethylenetriamine penta-acetic acid)] are the most accurate to assess renal function.” Thus, the need for CL measurements in liver failure is clear, but current radiometric methods are inadequate for this purpose [2, 4, 6, 8, 9].

In this study, Tk-GV CL values will be obtained for ten liver failure patients most of whom (8/10) have ascites. Investigated here is whether the Tk-GV models overcome the need for CL -value correction factors and maintain good quality even in the presence of ascites. In prior published work, the Tk-GV method has been applied to 4-h data using $^{99\text{m}}\text{Tc}$ -DTPA and ^{169}Yb -DTPA [13]. Tk-GV results provide quality control for each individual CL -value. The current paper is a first application of Tk-GV to ^{51}Cr -EDTA data, and to sampling data extended to 24-h. There is no a priori guarantee that Tk-GV will either usefully fit an untried pharmaceutical or 24-h data so testing is needed. The Tk-GV results are contrasted with those from mono-exponential (E1) fitting. E1 models fit to this new data are, from prior work, fairly certain to be problematic [13], especially in ascites [2, 4, 6, 8, 9]. Thus, how poorly E1 performs for our 24-h data is also of interest.

Intravenous (IV) bolus-injected inert markers clear into the interstitium as well as in the urine. Consequently, CL is slightly faster than glomerular filtration rate (GFR) [3, 5, 13], e.g. $CL \approx 1.076 GFR$ [5]. After IV bolus injection, CL estimates from multiple plasma samples are calculated from the area under the plasma marker disappearance curve (AUC) from time equals zero to infinity, i.e. $CL = \text{dose}/AUC$. AUC has units of concentration min. and marker (e.g. drug) systemic exposure time is plasma clearance (CL) related [1, 13]. Even the most minimalistic technique for estimating AUC , e.g. numerical integration [5], requires the use of a curve fit to extrapolate concentrations from the time of last sample to infinite time. In this paper, the E1 and Tk-GV curve fit methods for finding AUC are tested to determine the accuracy of CL from curve fitting of 4- versus 24-h data for ten ^{51}Cr -EDTA concentration curves. A key issue explored is how well clearances determined only from 4-h data predict the results determined from 24-h data. When the 4-h CL curve does a good job of predicting the 24-h concentrations, the model extrapolates the late-time

behaviour well, and the *AUC* values give accurate clearances. Conversely, if the 4-h *CL* curve fits do not predict the 24-h concentrations, then the ability of the fitting procedure to predict accurately the late-time behaviour is in question and the clearance values predicted from the method are suspect.

On a $\ln E1$ versus t plot, $\ln E1$ models are, of course, straight lines. However, let $C_{obs}(t)$ be the observed concentrations. Then, on a $\ln C_{obs}(t)$ versus t plot, the data are curved; thus $E1$ models do not fit data well. This same trend, i.e. curvature, is also seen in a plot of $\ln C_{obs}(t)$ versus $\ln E1$, and the Pearson r -value is less than 1. Indeed, from $\ln C_{obs}(t)$ versus $\ln E1$, the best R^2 -value that can be achieved by a linear fit (i.e. an $E1$ model of any kind, fit to any subset of the data) is the Pearson r -value squared, i.e. r^2 , as this is the limitation of the data's linearity. To achieve a better fit and to gain better predictability, one needs to use curves that follow the curvature of the $\ln C_{obs}(t)$ versus t plot. As we shall see, such a curve is the Tk-GV model. If such a model successfully follows the data's curvature, the observed log concentrations, $\ln C_{obs}(t)$, will be much better correlated with the model predictions. In other words, in a plot of $\ln C_{obs}(t)$ versus $\ln C_{Tk-GV}(t)$, the relationship should be almost perfectly linear, unlike the $\ln C_{obs}(t)$ versus $\ln C_{E1}(t)$ plots that show curvature and thus imperfect correlation.

Materials and methods

The patients are part of a research project comparing methods of assessing renal function in cirrhosis approved by the Royal Free Hospital Research Ethics Committee. Thirteen patients with cirrhosis under assessment for liver transplantation had ^{51}Cr -EDTA bolus IV studies. Of these, three patients did not have plasma samples drawn at various times, e.g. at 12- or 24-h, and were discarded. Of the remaining ten patients, four had hepatitis C-related cirrhosis (one also with hepatocellular carcinoma), three had alcoholic cirrhosis, two had primary biliary cirrhosis, and one had non-alcoholic, fatty liver disease-associated cirrhosis. Eight were men and two women: eight were white, one black and one South Asian Indian. The median weight was 79.5 kg (range 51.3–105.8), median serum bilirubin was 66 mmol/l (range 13–390), median serum albumin was 35 g/l (range 26–40) and median INR was 1.7 (range 1.5–2.4). Hepatic encephalopathy was present in eight and ascites in eight. The patients had 12–16 plasma samples drawn from 5-min to 24-h. Their concentration curves were fit with Tk-GV and $E1$ models and *CL* results were compared using Passing-Bablok fitting and paired (correlated) samples t -tests. A minimum of two samples determines an $E1$ solution, and fits to data from 5-min to 4-, 12- and 24-h are examined here. Also examined is a

recommended clinical method of obtaining $E1$ functions from plasma samples at 2- and at 4-h [2]. Exponential models have uniform concentrations (i.e. are well mixed, aka, instant mixing) in one or more partial volumes. For Tk-GV fitting, a minimum of four samples are needed. The GV concentration, $C(t)$, model used for Tk-GV fitting is $GV = C(t) = Kt^{\alpha-1}e^{-\beta t}$, where $C(t)$ is the marker concentration, α is the dimensionless volume of distribution scaling factor with $V = \alpha CL/\beta$, β is the renal elimination rate constant, K is a constant, and the mean residence time $MRT = \alpha/\beta$. The Tk-GV method uses least relative error of *CL* as a minimization target (see the Appendix) and is more robust than GV fitting with least-squares regression. The fit results for well-posed GV *CL*-values ($0 < \alpha \leq 1$, $\beta > 0$, and fits from 5 or 10 min to at least 3-h of data) are extensively described elsewhere [13]. In specific, the $0 < \alpha \leq 1$ constraint is *only* observed with Tk-GV fitting and does not occur with least-squares GV fitting. A Tk-GV model of an inert marker is a poorly mixed system with a concentration gradient such that the physical volume of the system is factor of α times smaller than the *virtual* volume (where the latter is CL/β). Moreover, the dynamic equilibrium concentration gradient is established by renal function and disappears, i.e. $\alpha \rightarrow 1$, only when renal function approaches zero [13].

Passing-Bablok fitting is used here for comparison of methods to provide unbiased linear regression slope and intercept [7]. The Passing-Bablok method includes the cusum test for linearity [7], which is useful for comparison of methods because it detects nonlinear relationships. Cusum $p > 0.1$ does not suggest nonlinearity, $0.05 < p < 0.1$ suggests nonlinearity, and $p < 0.05$ is highly suggestive of nonlinearity. Spearman rank correlation is used once for extracting a correlation in a slightly nonlinear relationship. Paired (i.e. two, correlated) samples t -testing is used here for comparison of methods using all the data. Two-sample t -testing is also applied to (paired) z -statistics from Fisher r - z transformation for detecting improved correlation. This transformation changes the otherwise non-normally distributed correlation coefficients (r) to more normally distributed z -statistics, using the inverse hyperbolic tangent function, $z = \text{atanh}(r)$. $C_{obs}(t)$ versus $\ln(t)$ plots are used to explain modelling effects. However, for calculating model fit quality correlations, we use the logarithm of both the observed concentrations and the predicted concentrations from the $E1$ and Tk-GV models. To test how linear the $\ln C_{obs}(t)$ versus $\ln C_{Model}(t)$ relationship is, one simply calculates its Pearson r value. (The correlation coefficient of determination, R^2 , is then the Pearson r -value squared, i.e. r^2 .) The test for ascites looks at the trend of fit quality, r , versus V/W , where V is the volume of distribution (ml) of the Tk-GV model, and W is the patient mass in kilograms. A model r -value that degrades with increasing ascites (as indexed to V/W) suggests a poorly behaved model. If the

model r -value is unchanged by increasing ascites, the model is relatively bulletproof.

Results

Figure 1 shows plots of 24-h concentrations of ^{51}Cr -EDTA in counts per ml min, $C(t)$, versus the logarithm of time, t , in min. Figure 1 plots show the highest and lowest $CL(\text{Tk-GV})$ cases (79.7 and 11.4 ml/min) in this series. These plots suggest an improvement in fit quality for 24-h data fits using Tk-GV functions compared to the E1 functions. $C(t)$ versus $\ln(t)$ plots roughly linearize the early-time $C(t)$. Figure 1 demonstrates that the exponential fit assumption of a constant concentration at time zero causes underestimation of the early concentrations of actual data. Let us define late-time $C(t)$ as being after the concentration curve becomes nonlinear in $\ln(t)$, i.e. asymptotic to the $C(t)=0$ axis. Figure 1a demonstrates that when an exponential fit includes the late-time concentrations, late-time concentrations are accurately fit. Figure 1b illustrates that when an exponential fit does not include late-time concentrations, the late-time concentrations are underestimated.

For several reasons, the correlations (r) used for comparison of methods are calculated from the observed versus predicted logarithms of concentration values. These r -values (Table 1) are only indirectly related to r -values

calculated without taking logarithms (e.g. the R^2 in Fig. 1). Comparing methods, the improvement in correlation to logarithm concentration of $\ln C_{\text{obs}}(t)$ and $\ln C_{\text{Model}}(t)$ was highly significant ($p < 0.0001$) for Tk-GV functions compared to the E1 functions using paired samples t -testing of the z -values from $z = \text{atanh}(r)$. Moreover, the r -values are better for Tk-GV than for E1 fits in each of the ten cases (Table 1). With a median 24-h Tk-GV fit of $r = 0.998$, there is little unexplained variance in the logarithm concentration data models ($1 - r^2 = 0.4\%$). This gives us confidence that extrapolation from 1,440 min to infinite time and from 5 min to zero time is accurate. Next, one uses Tk-GV fits to estimate the percentage of area under the curve (AUC) of concentration versus time not directly under the samples. Not surprisingly, there is a lesser percentage of extrapolated AUC for higher CL (e.g. Table 1, case 3, 5.4%), and a higher percentage for lower CL (case 10, 48.3%), and it takes longer than 24-h to clear ^{51}Cr -EDTA in renal failure. However, the goodness of fit for Tk-GV allows us to have confidence that 24 h of AUC data is long enough to calculate accurate CL -values. An indication of $CL(\text{Tk-GV})$ precision is given as standard deviation (SD) of CL , which values range from 0.4 to 3.5 ml/min in Table 1. The Tk-GV method produces these estimates during processing. These estimates correspond to 95% confidence intervals for errors of 24-h CL -values that range from ± 0.8 to ± 6.9 ml/min.

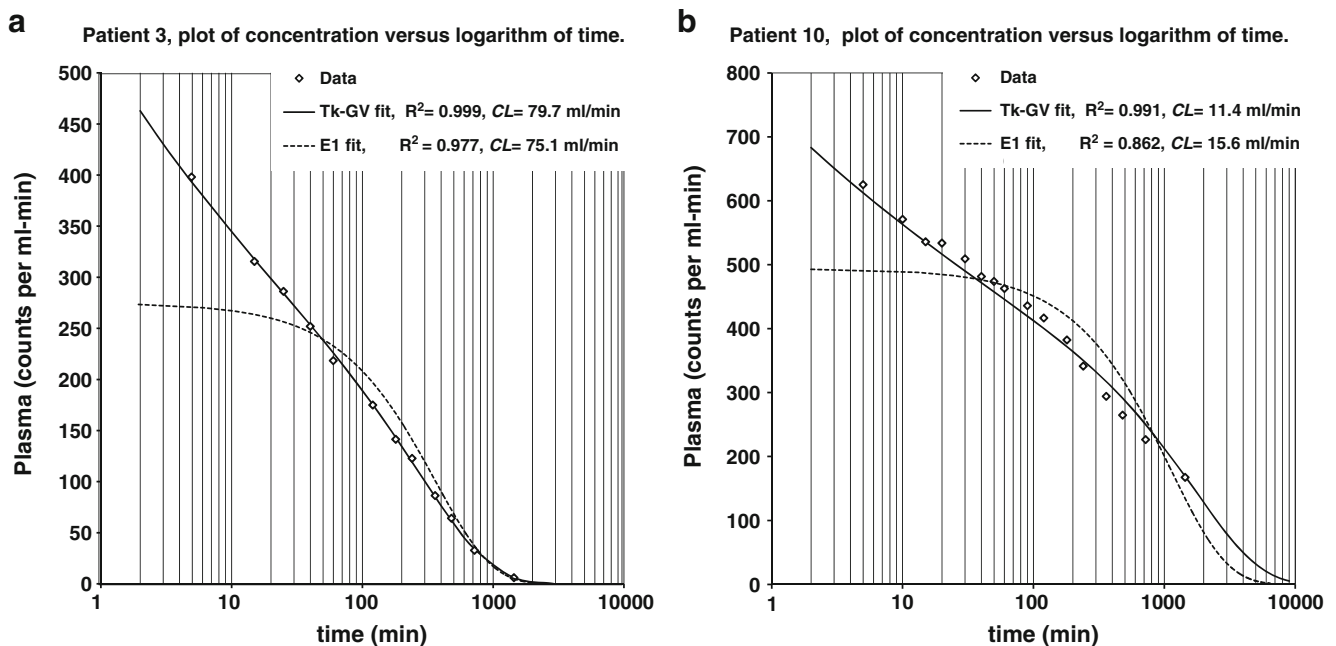


Fig. 1 **a** Plot of 24-h concentrations of ^{51}Cr -EDTA in counts per ml min, $C(t)$, per logarithm of time, t , in min for patient 3's data. For patient 3's 24-h data, $R^2(\text{Tk-GV}) = 0.999 > R^2 = 0.977$ from a one exponential term model (E1). The $CL(\text{Tk-GV})$ for patient 3 is the highest CL in this series (79.7 ml/min). **b** Plot of 24-h concentrations,

$C(t)$, per logarithm of time, t , for patient 10's data, having the lowest $CL(\text{Tk-GV})$ in this series (11.4 ml/min). Right-hand areas contribute more to AUC than those on the left do, for this plot type. For example in **b**, the AUC for the Tk-GV curve from 1,440 min to infinite time is 47.8% of the total area

Table 1 From 24-h fitting to logarithms of concentrations for Tk-GV, CL and V (l) ± 1 standard deviation (SD) error, V as percent of W (weight-kg), and percentage of AUC from extrapolation from left tails (0 to 5 min) and right tails (1,440 min to infinite time). Also shown are correlation coefficients, r , for Tk-GV and E1 fitting

Case	$CL \pm 1$ SD (ml/min)	$V \pm 1$ SD (l)	V/W (%)	AUC of tails (%)	Tk-GV r	E1 r all fits
3	79.7 \pm 0.4	29.7 \pm 0.2	34.9	5.4	1.000	0.988
5	76.5 \pm 1.6	38.9 \pm 1.2	45.2	13.0	0.999	0.933
6	56.5 \pm 0.9	21.3 \pm 0.6	29.6	6.8	0.998	0.975
9	46.5 \pm 0.8	18.6 \pm 0.3	22.8	6.9	0.999	0.977
4	45.8 \pm 0.8	21.9 \pm 0.4	35.6	9.0	0.999	0.971
12	42.5 \pm 2.1	26.9 \pm 0.9	39.7	13.7	0.995	0.960
13	36.4 \pm 3.1	41.9 \pm 1.8	39.6	29.3	0.988	0.937
8	29.9 \pm 3.5	60.8 \pm 4.7	59.9	45.5	0.998	0.848
11	21.2 \pm 0.8	17.7 \pm 0.4	34.7	19.5	0.998	0.970
10	11.4 \pm 0.9	23.4 \pm 0.6	30.4	48.3	0.994	0.929

As per the “Materials and methods” section, $\ln C$ from E1 fit equations from any sample subsets have at best a quality of fit to $\ln C_{obs}(t)$ of the correlation coefficient evaluated at all the sample times (median as $r=0.965$, see Table 1). The correlation of r -values from E1 24-h data fits with V/W (Table 1) is significantly negative, $r[r(E1), V/W] = -0.823, p=0.0034$. However, the correlation of r -values from 24-h Tk-GV fits to the data with the V/W from the same Tk-GV model is uncorrelated, $r[r(Tk-GV), V/W] = -0.084, p=0.8165$.

Changing sample subsets can produce different CL -values, especially for E1, as follows. With reference to Table 2 and Fig. 2a, the 4-h (vertical axis) versus 24-h (horizontal axis) $CL(Tk-GV)$ plot yielded no significant Passing-Bablok departure from a line slope of 1 or an intercept of 0. This means that 4-h Tk-GV clearances

unbiasedly predict 24-h Tk-GV clearances. However, the R^2 -value for this relationship is only 0.738, and the more powerful paired samples t -test shows a (borderline) significant bias ($p=0.04$). This borderline significant mean difference is 8.6 ml/min greater CL measured for the 4-h data than for the 24-h data. Much of the variance for predicting 24-h CL from 4-h data is not related to the method of calculation but to the data themselves, i.e. from propagation of errors. For example, the furthest 4-h versus 24-h $CL(Tk-GV)$ outlier of Fig. 2a, 64.4 versus 29.9 ml/min, is only a near outlier at 2.6 interquartile ranges (IQR) and would not usually require explanation. To decrease the 4- versus 24-h $CL(Tk-GV)$ difference from 11.5 to 3.9 ml/min (1 SD), one carries the fits out to 12-h (instead of 4-h) of data when comparing to the 24-h CL . Note (Table 2) that for 12- versus 24-h, there is no significant departure from a line

Table 2 Tk-GV and E1 line fit tests for slope, $m=1$, and intercept $b=0$ and test for significance of different mean values of CL

Passing-Bablok fit lines for (hr) = 4, 12, 24, 2&4	Parameter values		95% CI	CI	Paired t -test (Δ in ml/min)		R^2
	m	b	Low to high	Significance	Probability	Significance (Δ)	
Tk-GV(4) = $m \cdot Tk-GV(24) + b$	$\left[\begin{matrix} m & 0.971 \\ b & 6.7 \end{matrix} \right.$		0.568 to 1.50 -15.4 to 28.5	NS NS	0.0416	B (8.6)	0.738
Tk-GV(12) = $m \cdot Tk-GV(24) + b$	$\left[\begin{matrix} m & 0.986 \\ b & 3.3 \end{matrix} \right.$		0.795 to 1.15 -5.0 to 12.8	NS NS	0.0337	B (3.1)	0.968
E1(4) = $m \cdot E1(24) + b$	$\left[\begin{matrix} m & 1.56 \\ b & 5.2 \end{matrix} \right.$		1.16 to 9.77 -347 to 17.4	S NS	0.0001	S (32.1)	0.738
E1(12) = $m \cdot E1(24) + b$	$\left[\begin{matrix} m & 1.11 \\ b & 3.0 \end{matrix} \right.$		0.98 to 1.45 -10.7 to 7.63	NS NS	0.0002	S (9.1)	0.951
E1(4) = $m \cdot Tk-GV(4) + b$	$\left[\begin{matrix} m & 1.31 \\ b & 6.5 \end{matrix} \right.$		1.04 to 1.62 -8.6 to 18.9	B NS	<0.0001	S (23.9)	0.928 ^a
E1(24) = $m \cdot Tk-GV(24) + b$	$\left[\begin{matrix} m & 0.850 \\ b & 6.0 \end{matrix} \right.$		0.417 to 0.940 1.0 to 26.6	S B	0.8386	NS (0.4)	0.948
E1(2&4) = $m \cdot Tk-GV(24) + b$	$\left[\begin{matrix} m & 1.01 \\ b & 16.4 \end{matrix} \right.$		0.626 to 1.59 -7.0 to 36.0	NS NS	0.0008	S (20.7)	0.740

CI confidence interval, R^2 is coefficient of determination, S significant ($p < 0.01$), NS not significant ($p > 0.05$), B borderline significant ($0.01 < p < 0.05$)

^a Nonlinear, $0.05 < p < 0.1$ from cusum. However, R^2 (0.928) and the Spearman rank coefficient squared (0.929) are similar

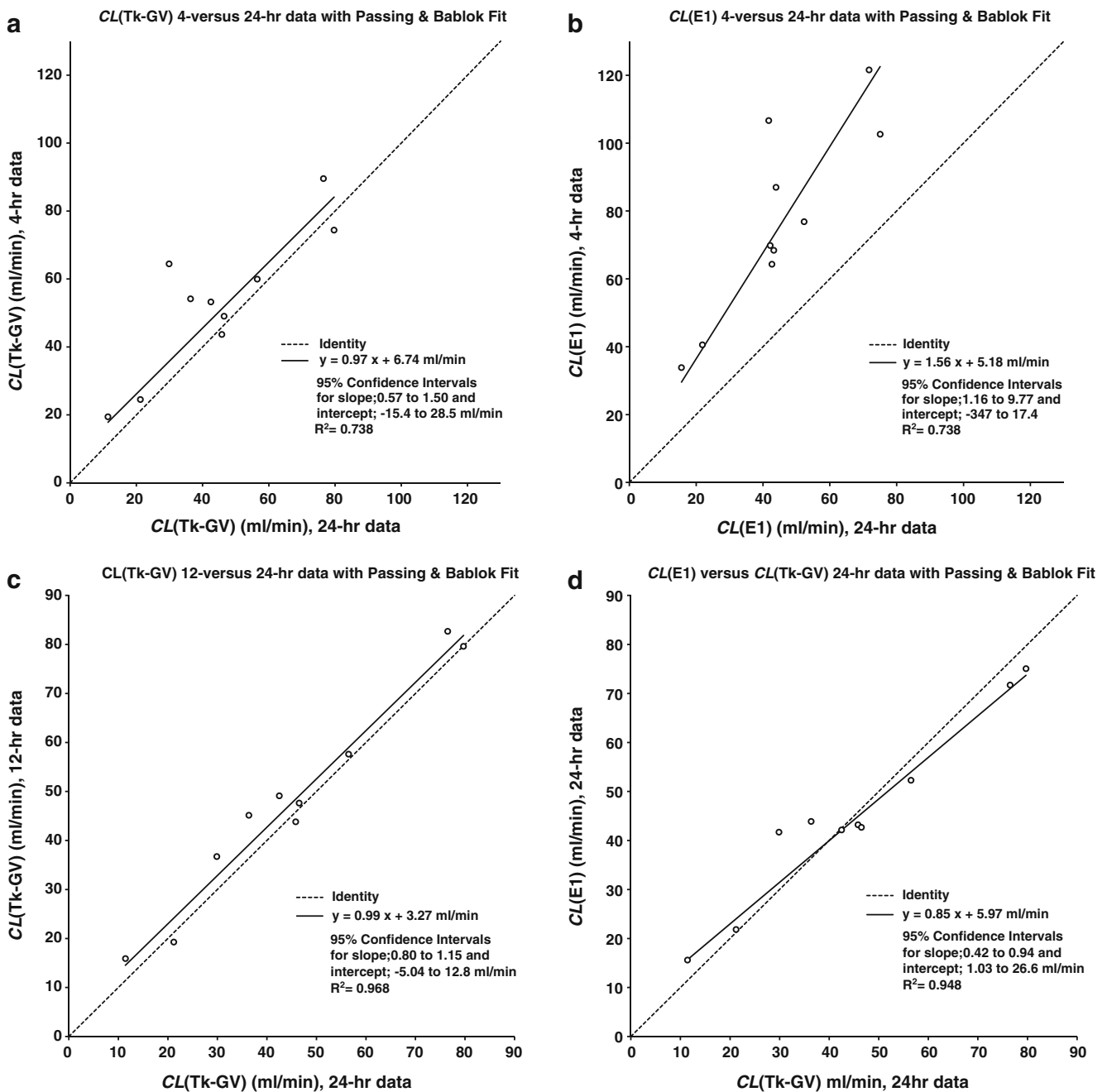


Fig. 2 **a** The Tk-GV plasma clearance method, $CL(Tk-GV)$, was applied to 4- and 24-h data and plotted. When this is fit with a Passing-Bablok regression line, there is no significant departure from unity slope and no significant y -intercept. **b** The 4-h data $CL(E1)$, plotted against 24-h $CL(E1)$, has a slope significantly >1 , i.e. the 24-h $CL(E1)$

values are overestimated by 4-h $CL(E1)$ calculations. **c** $CL(Tk-GV)$ for 12- and 24-h data and no significant departure from a slope of 1 and an intercept of zero. **d** Even for 24-h data, $CL(Tk-GV) \neq CL(E1)$, with both the slope $\neq 1$ and the intercept $\neq 0$ of the Passing-Bablok linear fit

slope of 1 or an intercept of 0 and the R^2 improves (0.968). However, the t -test, 3.1 ml/min mean bias, although small, is still (borderline) significant ($p = 0.03$).

Significantly poorer results were obtained from the 4-h versus 24-h E1 fitting (see Table 2). Figure 2b shows these results plotted with a regression slope of 1.56, i.e. the 4-h CL -values are significantly larger than the 24-h CL -

values with a very significant ($p = 0.0001$) mean difference of 32.1 ml/min. However, the R^2 value for this plot is 0.738, whereas the R^2 value (or Spearman rank sum squared, r_s^2) for 4-h $CL(Tk-GV)$ versus $CL(E1)$ data would be significantly better, i.e. 0.928 ($r_s^2 = 0.929$). That the R^2 values of 4- versus 24-h $CL(Tk-GV)$ and 4- versus 24-h $CL(E1)$ are equal to 3 decimal places (i.e. 0.738), and 4-h $CL(Tk-GV)$

and $CL(E1)$ agree better with each other (0.928) than either 4-h CL agrees with its corresponding 24-h result (i.e. 0.738) appears to confirm error propagation as explaining much of the variance for predicting 24-h CL from 4-h data.

When the sample times included in the fit are increased to 12 h, then $CL(E1-12-h) > CL(E1-24-h)$ in all cases. The paired t -test for this is highly significant ($p = 0.0002$, mean difference 9.1 ml/min), with an improved R^2 (0.951), (see Table 2). From the 24-h data (Fig. 1d), the plot of $CL(Tk-GV)$ versus $CL(E1)$ has a significantly biased slope (0.85) and borderline significant intercept (5.97 ml/min, $0.01 < p < 0.05$). Note that from Table 2, the t -test $p = 0.8386$ mean difference of 0.4 ml/min is not significant. In other words, on the average, $CL(Tk-GV-24-h)$ values did not differ from $CL(E1-24-h)$ values, but, for low CL -values, $CL(E1) > CL(Tk-GV)$, and for high CL -values, $CL(E1) < CL(Tk-GV)$.

The slope intercept (E1) 2- and 4-h plasma sample method results follow. The Passing-Bablok regression slope of $CL(E1-2- \& 4-h)$ versus $CL(Tk-GV-24-h)$ has no bias detected for slope (1.01) and intercept (16.4 ml/min). However, the intercept is larger than for $CL(Tk-GV-4-h)$ versus $CL(Tk-GV-24-h)$ (6.7 ml/min). Indeed, paired t testing (Table 2) shows the mean bias (20.7 ml/min) to be highly significant ($p = 0.0008$) for $CL(E1-2- \& 4-h)$ versus $CL(Tk-GV-24-h)$. This compares to the more moderate mean bias (8.6 ml/min) of borderline significance ($p = 0.0337$) for the $CL(Tk-GV-4-h)$ versus $CL(Tk-GV-24-h)$ plot. The Passing-Bablok regression of $CL(E1-24-h)$ (vertical axis) versus $CL(E1-2- \& 4-h)$ (horizontal axis) has a significantly lesser slope ($p < 0.01$) than 1, i.e. 0.65. However, both $CL(E1-24-h)$ and $CL(E1-2- \& 4-h)$ have r -values for fitting the logarithms of concentrations that are very significantly poorer (z -statistic t -test $p < 0.0001$) than $CL(Tk-GV-24-h)$.

Discussion

The Tk-GV models have a less obvious dependence on sampling-time effects than E1 fits. They also have less bias for extrapolation and fit the data very significantly better ($p < 0.0001$). Of some concern is that the predictions of 24-h CL from 4-h CL data were imprecise ($R^2 = 0.738$). This lower than optimal coefficient of determination is partly due to a restricted range of CL -values from examining low renal function patients only, may partly be due to biological fluctuation of CL in time and is due to the propagation of errors of measured concentrations, which in turn is due to errors in sample measurements, e.g. see [2]. In early prospective clinical experience, $CL(Tk-GV)$ has been implemented for four-sample 4-h ^{99m}Tc -DTPA, with some notable features being that the ease of use, stability of the solutions and that the error calculations help to improve sampling technique and inspire confidence in the results. Some of the

sources for error are easily addressed. For example, extravasation in the injection site is reduced by applying the flush-bolus technique and checked for by imaging of the injection site [12]. Whatever the causes for error, use of Tk-GV has the advantage of increased accuracy for extrapolated CL (defined as lack of bias) while having precision (defined as R^2 for CL extrapolation) as good as or better than that of E1 fitting.

Fractal structures have been postulated as causing a gamma variate (GV) shape for inert substance CL -curves [13]. However, it is only when a GV is found using a CL relative-error minimizing technique that GV (1) accurately predicts the late-time concentration and (2) consistently forms a model with a dynamic concentration gradient established by renal function. The results here suggest that the 24-h $CL(Tk-GV)$ values are a standard against which to judge clearance, because of the very good quality of all of the curve fits (median $r = 0.998$) (see Fig. 1 and Table 1). Moreover, prediction of 24-h $CL(Tk-GV)$ from 4- and 12-h $CL(Tk-GV)$ was good, suggesting that 24-h Tk-GV fits should extrapolate concentration correctly at even later times. In particular for six statistical tests, $CL(Tk-GV)$ extrapolation was unbiased for four tests and borderline biased (i.e. $0.01 < p < 0.05$) for two, whereas $CL(E1)$ was significantly biased in three of six tests. The 4-h $CL(Tk-GV)$ versus $CL(E1)$ (Table 2) is somewhat nonlinear (cusum probability, $0.05 < p < 0.1$ [7]) and the clinically used slope intercept method with 2- and 4-h plasma samples differs from 24-h $CL(Tk-GV)$ by a constant (mean difference 20.7 ml/min, $p = 0.0008$), so there is no “quick-fix” linear transformation of $CL(E1)$ values to estimate $CL(Tk-GV)$. This is discordant with the literature suggesting that $CL(E1-2- \& 4-h)$ may be corrected by multiplying by 0.87, i.e. the Chantler correction factor, and does not suggest subtracting a constant [2]. However, it is easy enough to see how such an impression could arise as the Passing-Bablok regression of $CL(E1-24-h)$ (vertical axis) versus $CL(E1-2- \& 4-h)$ (horizontal axis) only has a significant slope (0.65, $p < 0.01$ of slope =1) and an insignificant intercept. However, both $CL(E1-24-h)$ and $CL(E1-2- \& 4-h)$ have r -values for fitting the logarithms of concentrations that are very significantly poorer (z -statistic t -test $p < 0.0001$) than $CL(Tk-GV-24-h)$. Thus, subtraction of a 16.4 (or 20.7) ml/min constant from the $CL(E1-2- \& 4-h)$ values may be the more accurate correction, at least for our patients with ascites [and if one accepts that $CL(Tk-GV-24-h)$ is a gold standard].

A final caveat is that eight of ten patients had ascites. Ascites has been associated with increased CL for compartmental models by 16–20 ml/min [4, 6, 9] and is considered by some to relatively contraindicate CL testing due to gross inaccuracy [2]. However, ascites seems to have had little effect on the $CL(Tk-GV)$ -values here. This is significant and it needs to be emphasized that the Tk-GV

method is valid in patients with low *GFR* and with ascites. This comes about as the Tk-GV volumes of distribution (*V*) (and *V/W*, Table 1) are larger than usual (e.g. see [2]). However, that is an expected result for ascites (also see [8]), as follows. For a bolus clearance model, $MRT = V/CL$, where *MRT* is the mean residence time. If in that equation *CL* is held constant, then for a longer residence time, *V* must be larger, and for a good model, that is an expected outcome of ascites. *V/W* is our measure of ascites from the 24-h Tk-GV model. The correlation of *r*-values from E1 24-h data fits with *V/W* (Table 1) is significantly negative, $r[r(E1), V/W] = -0.823$, $p = 0.0034$. However, the correlation of *r*-values from 24-h Tk-GV fits to the data with *V/W* from the same Tk-GV model is insignificant, $r[r(\text{Tk-GV}), V/W] = -0.084$, $p = 0.8165$. For $r[r(E1), V/W]$, we have not caused a correlation by reusing parameters, because the only sharing between models is from the characteristics of the data themselves. For $r[r(\text{Tk-GV}), V/W]$, we have reused parameters, and despite this, there is no correlation. We conclude that using *V/W* (from 24-h Tk-GV) as an index of the severity of ascites, E1 fits degrade as ascites increases, and Tk-GV fit quality is not measurably affected. This finding confirms the observation that use of E1 (or E1 extrapolation) is problematic in ascites [4, 6, 8, 9] and presents a solution to this problem using Tk-GV. To inspect this in detail, let us examine the GV rate equation,

$$\frac{1}{C(t)} \frac{dC(t)}{dt} = \frac{\alpha - 1}{t} - \beta, \quad (1)$$

where $(\alpha - 1)/t$ is definite negative, is associated with tissue clearance, and gets slower and slower in time (it is proportional to $1/t$). $CL > GFR$ implies $\alpha \leq 1$, where $\alpha > 1$ is a common [8], but ill-posed solution for *CL* for GV fitting [13]. Here, using Tk-GV fitting, the entire range of α for our 24-h data is from 0.62 to 0.88, i.e. α is well behaved. So α does not change by much to accompany an increase of fluid volume. Here, a low $\alpha = 0.67$ coincides with the most inflated volumes of distribution as a percentage of patient mass 59.9% (case 8, Table 1).

Normally slow or pathologically slow clearance areas in the body include ascites or abdominal fluid, some adenomas and neoplasias, some chemotherapy, pleural or pericardial fluid, lymphatic circulation and lymphoedema, cerebrospinal fluid circulation, synovial fluid, circulation in aqueous humour, abscess, cellulitis, fibrous dysplasia, etc. The entire spectrum of slow tissue marker leakage rates is included in GV rate Eq. 1 because for all $t > (1 - \alpha)/\beta$, the rates of losses of marker into tissue are slower than renal loss. That is, tissue marker leakage rates converging to zero (i.e. to an infinite half-life) are an integral part of the GV model. By contrast, an infinite sum of exponential terms would be

needed to approximate the effect of the temporal spectrum of the single, gradually decreasing rate term of the GV model.

Why only compare Tk-GV to E1 fitting? Why not then test E2, E3, E4 or E_n (i.e. sums of exponential terms) in general? The reason is that a robust model is strongly desirable, and $E(n > 1)$ is not stable for regression analysis unless the number of samples is substantially greater than *n* [10, 13]. Although methods exist for stabilizing E2 models (e.g. see [11]), in general for regression, E_n is not robust, indeed ordinary least-squares GV function fitting is also not robust. However, GV fitting can be made robust by regularization (as in Tk-GV [13]). It is at best tedious and at worst impossible to obtain four (or more) stable parameter estimates with an unstable E2 (or higher) regression model. By contrast, a single attempt suffices to obtain the globally converged Tk-GV model's three parameters, with superior quality curve fits, better extrapolation to late-time concentrations from fits to early concentration data and using fewer samples [13]. For practical measurements, one strongly desires either a simple robust model (e.g. E1 with correction factors) or another robust model (e.g. Tk-GV with no need of correction factors). However, as shown in the results above (Table 2) the magnitude and the type of the correction factors needed for E1 *CL*-values are a function of the times used to obtain the samples. Even if it were clear how to correct E1 *CL*-values (and it is not), any such timing correction formulae would have empirical coefficients. For example, these formulae would not apply to both small and large mammals without allometric rescaling. Moreover, E1 fit quality degrades in ascites, rendering the utility of the model questionable. In summary, making "corrections" to an inherently flawed exponential term model is not optimal. The better approach using Tk-GV better fits the observed real-life data, without corrections needed.

Is 24-h a long enough data collection time to determine *CL* in renal failure ($CL < 15$ ml/min)? The results suggest that if we use E1 functions for fitting, the answer would be no. It is common to plot logarithm concentration versus time, which would linearize $\ln C(t) \approx -at + b$, i.e. an E1 function. In fact, note in Fig. 1 that *C(t)* at early times is approximately linear in $\ln t$, i.e. $C(t) \approx -a \ln t + b$. This latter plot type helps visually confirm the correlation results, in that E1 functions have the wrong shape and do not follow the concentration curves well. Adding more exponential terms does not solve the curve shape problem. Let us define late-time *C(t)* as being after the concentration curve becomes nonlinear in $\ln(t)$, i.e. asymptotic to the $C(t) = 0$ axis. Figure 1b demonstrates that when an exponential fit does not include late-time concentrations, the late-time concentrations are underestimated. This explains the overestimation of *CL* (from underestimation of *AUC*) for (all choices of) E1 early-time extrapolations to later-time fits in Table 2. However, with the better-fitting Tk-GV functions, the

extrapolation errors are not so severe, as suggested by extrapolation testing of early data. Using Tk-GV for the lowest *CL* value (11.4 ml/min, case 10, Fig. 1b), one predicts that it would take 6 days of data collection for the concentration to go below 1% of the concentration measured in the 5-min sample. Adding a “terminal” exponential to the 1-day data collection would underestimate the concentration at day 6, as exponentials go to zero too quickly to mimic late concentration. Adding another early exponential term to the Fig. 1 E1 fits would not remove the exponential fit assumption of a constant concentration at time zero causing underestimation of the early concentrations of actual data. On the other hand, finding the *AUC* is not especially challenging when the data collected include all the concentrations that contribute to them, and any arbitrary interpolative curve fitting, or numerical integration [5], will suffice for that purpose. Especially as relates to early-time only data collections, proper extrapolation is not as arbitrary as interpolation and must be validated by extrapolation testing, done here for Tk-GV. Thus, if a 4- or 12-h test is desired for finding *CL*, Tk-GV fitting appears to provide an accurate (i.e. relatively unbiased) method of predicting *CL* for 24-h, even in the presence of ascites.

Conclusion

CL(E1) values were dependent on sample time and the quality of fit was poor and degraded with increasing ascites, consistent with current opinion that *CL*(E1) is contraindicated in ascitic patients. *CL*(Tk-GV) was relatively more accurate and the good quality of fit was unaffected by ascites. *CL*(Tk-GV) was the preferred method for the accurate calculation of *CL* and was useful despite liver failure and ascites.

Conflicts of interest None.

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Appendix

Tikhonov regularization

For an overdetermined system of linear equations, $\mathbf{Ax}=\mathbf{b}$, the Tikhonov regularization (Tk) of this problem introduces the penalty function $\Gamma\mathbf{x}$ and seeks to find a solution that minimizes $\|\mathbf{Ax} - \mathbf{b}\|^2 + \|\Gamma\mathbf{x}\|^2$. This latter is the square of a norm of the residuals, $\|\mathbf{Ax} - \mathbf{b}\|^2$, plus the square of a norm of the product of the Tikhonov matrix, Γ , with the \mathbf{x} fit parameters (unknowns). The more general $\Gamma^T\Gamma$ regularizing

term is often, as it is here, replaced by $\lambda\mathbf{I}$, where \mathbf{I} is the identity matrix, and λ is a Lagrange (i.e. constraint) multiplier, also commonly called the shrinkage, Tikhonov or damping factor.

A constraint on $\ln K$

A most common constraint for regression is to require the fit function to pass through the data mean point [aka the centroid, (\bar{x}, \bar{y})]. Because the logarithm of concentrations is the more homoscedastic quantity, it is common to fit the logarithms of marker concentrations rather than the concentrations themselves. Thus, for the Tk-GV method, the GV function is written $\ln C = \ln K + (\alpha - 1) \ln t - \beta t$, where the constant term $\ln K$ need not be independent, but can be determined from the other fit parameters using a mean value constraint. Taking averages over the data

$$\ln K = \overline{\ln C(t)} - (\alpha - 1)\overline{\ln t} + \beta\bar{t} = \bar{b} - (\alpha - 1)\bar{a}_1 + \beta\bar{a}_2, \tag{2}$$

such that \bar{b} , \bar{a}_1 and \bar{a}_2 are data constants, where \bar{b} is the mean value of the logarithms of the concentrations, \bar{a}_1 is the mean of the logarithms of the sample times and \bar{a}_2 is the mean of the sample times. Then, Eq. 2 is used to remove K from the formula for *CL*, and an expression is derived for the errors in *CL* with only α and β as independent parameters, as follows

$$CL = \frac{D}{AUC} = \frac{D\beta^\alpha}{K\Gamma(\alpha)} = \frac{D\beta^\alpha}{\exp[\bar{b} - (\alpha - 1)\bar{a}_1 + \beta\bar{a}_2]\Gamma(\alpha)}, \tag{3}$$

where the gamma function, $\Gamma(x) = \int_0^\infty t^{x-1}e^{-t}dt$.

Error propagation and the error adaptive equation

One applies error propagation (Taylor series expansion) to the right-hand *CL* expression of Eq. 3 with respect to α and β yielding

$$\begin{aligned} \left(\frac{s_{CL}}{CL}\right)^2 &= s_\alpha^2(\bar{a}_1 + \ln\beta - \Psi(\alpha))^2 + s_\beta^2\left(\frac{\alpha}{\beta} - \bar{a}_2\right)^2 \\ &+ 2s_{\alpha\beta}(\bar{a}_1 + \ln\beta - \Psi(\alpha))\left(\frac{\alpha}{\beta} - \bar{a}_2\right). \end{aligned} \tag{4}$$

where $\Psi(\alpha)$ is the digamma function of α and $\Psi(\alpha) = d[\ln \Gamma(\alpha)]/d\alpha = \Gamma'(\alpha)/\Gamma(\alpha)$, the subscripted s variables are the standard deviations of the subscripted quantities and $(s_{CL}/CL)^2$ is the squared coefficient of variation CV^2 of *CL*. Minimizing the right-hand side of Eq. 4 as a function of the shrinkage, λ , selects a λ value that produces the *CL* value with the smallest relative error achievable. Also, minimizing the relative error in *CL* is indispensable for making reliable measures of *CL* when *CL* is small.

The squared coefficient of variation, CV^2 , of the individual volumes of distribution, i.e. $(s_V/V)^2$, is calculated from the SDs produced from minimizing Eq. 4 substituted into

$$\begin{aligned} \left(\frac{s_V}{V}\right)^2 &= s_\alpha^2 \left(\frac{1}{\alpha} + \bar{a}_1 + \ln \beta - \Psi(\alpha)\right)^2 + s_\beta^2 \left(\frac{\alpha}{\beta} - \frac{1}{\beta} - \bar{a}_2\right)^2 \\ &+ 2s_{\alpha\beta} \left(\frac{1}{\alpha} + \bar{a}_1 + \ln \beta - \Psi(\alpha)\right) \left(\frac{\alpha}{\beta} - \frac{1}{\beta} - \bar{a}_2\right). \end{aligned} \quad (5)$$

Equation 5 is from application of the propagation of error formula to the substitution of Eq. 3 into $V=(\alpha/\beta)CL$.

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