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^{51}Cr-EDTA plasma clearance in children

One, two, or multiple samples?

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Abstract

Plasma disappearance curves using multiple blood samples are a recognized reference method for measuring glomerular filtration rate (GFR). However, there is no consensus on the protocol for this type of measurement. A two-compartment model is generally considered acceptable for the mathematical description of the concentration–time decay curve. The impact of the fitting procedure on the reported GFR has not been questioned.

We defined 8 different fitting procedures to calculate the area under the curve, and from this area under the curve, the GFR. We applied the 8 fitting methods (all considering a full concentration–time curve) on the multiple sample data (8 samples) of 20 children diagnosed with Duchenne muscular dystrophy. We evaluated the effect (variability) on the reported GFR from the different fitting methods and compared these results with GFR-values calculated from late samples only (samples after 120 minutes) and from one-sample methods.

In 6 out of 20 cases, the fitting methods on the full concentration–time curve resulted in very different reported GFR-values, mainly because some methods were not able to fit the data, or methods resulted in GFR-values ranging from 0 to 120 mL/min. The reported GFR-result therefore strongly depends on the fitting method, making the full concentration–time method less robust than expected. Compared with a consensus reference GFR, the late sample models did not show fitting issues and may therefore be considered as more robust. Also the one-sample methods showed acceptable accuracy.

The late sample methods (using 3 time-points) provide robust and reliable methods to determine GFR.

Abbreviations: %CV = coefficient of variation, ^{51}Cr-EDTA = chromium-51 labeled ethylene diamine tetra-acetic acid, AUC = area under the curve, BM = Bröchner-Mortensen (correction), BSA = body surface area, CCC = concordance correlation coefficient, CKD = chronic kidney disease, GFR = glomerular filtration rate, GFR_S = slow GFR, ID = identity, mSI = modified Slope-Intercept, mS-NLLS = modified split scenario for unweighted non-linear least squares, mS-NLLS-w = modified split scenario for weighted non-linear least squares, NLLS = non-linear least-squares, NLLS-w = weighted non-linear least-squares, RMSE = root mean square error, SD = standard deviation, SI = slope-intercept, S-NLLS = split scenario for unweighted non-linear least-squares, S-NLLS-w = split scenario for weighted non-linear least-squares.

Keywords: chromium-51 labeled ethylene diamine tetra-acetic acid plasma clearance, children, multiple samples

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1. Introduction

Many centers are still reluctant to perform direct glomerular filtration rate (GFR) measurements in children. It has mainly to do with the discomfort for the child, because of the intravenous injection of the filtration marker with subsequent blood sampling. However, GFR is generally accepted as the best indicator for renal function and GFR may be reduced before the onset of symptoms of renal failure, allowing early diagnosis and therapeutic interventions in children at risk. Although inulin clearance with continuous infusion is considered as the gold standard, it is not very practical in children. Urinary clearances (whatever the marker) are difficult to perform in children, especially in the very young. Plasma clearance of exogenous markers (e.g., chromium-51 labeled ethylene diamine tetra-acetic acid [^{51}Cr-EDTA] or iohexol) have replaced the gold standard method with acceptable accuracy and precision.[1,2] Standardization of the procedure is still lacking, but ongoing research has led to a consensus procedure in which the plasma disappearance curve after a bolus injection of a glomerular tracer is described by a double exponential decay curve $c(t) = A_1 \times \exp(-B_1 \times \text{time}) + A_2 \times \exp(-B_2 \times \text{time})$. The first (or early) exponential is corresponding to the distribution of the marker in the body. The second (or late) exponential is slower and is depending on the excretion of the marker by the kidney. By fitting this decay curve, the coefficients can be determined and from these, the area under

the curve (AUC) = $A_1/B_1 + A_2/B_2$. The GFR then equals the dose or activity of the injected tracer divided by the AUC. To accurately fit the curve, the method requires multiple blood sampling (8–10 samples) and therefore it is not frequently used in children.[3–5] Simplified methods have been proposed for clinical routine in children.[6,7] One method is based on the determination of only the late samples (at least 2 samples are required, e.g., at 2 and 4 hours after intravenous injection in healthy subjects, later in children with chronic kidney disease [CKD]). This slope-intercept method allows the calculation of the AUC, and thus of the GFR. Details of this method are still a matter of debate, more precisely, the number of blood samples to be taken, the time-points at which they should be taken, the correction method for having neglected the early exponential[8] and the lack of quality control in case only 2 samples are used (as statistical error estimation is only possible when at least 3 samples are used in the calculation). A further reduction of the number of samples to only one sample, is an entirely empirical method, but is easiest from a practical point of view.[9–12] Again, the details of the one-sample methods are still a matter of debate, including the discussion on the choice of the time-point, the accuracy of the method, and the total lack of quality control. Several algorithms are available, which are also valid for adolescents and adults. The one-sample method may lose accuracy at low GFR, or the time-point chosen should be shifted to higher values.[13]

The aim of the current research study was to evaluate the reproducibility of the calculated AUC (and thus the GFR) using different fitting methods for the full concentration–time curve, which is considered the reference standard method. In fact, all simplified methods (based on a few late samples, or only one sample) are developed against the GFR determined from the reference standard procedure. The fitting procedure has not been questioned as a possible source of error. We have recently demonstrated the variability of reported GFR-results due to the choice of the fitting procedure in older adults,[14] with moderately reduced GFR. In the current study, we wanted to investigate whether this variability due to the fitting method also exists in the high GFR-range. After establishing a consensus on the reported "reference standard" GFR, based on the quality and reproducibility of the reported GFR-value, we also evaluated the accuracy of the simplified methods: the method based on late samples combined with different correction methods for the absence of the early component.[15–19] and the method based on one sample, using different algorithms.[11,13,14]

2. Methods and participants

2.1. Participants

Data were available from Leuven University Hospital. The study was approved by the Institutional Ethical Board of UZ Leuven. Consent forms were signed by parents of participants ≤17 years of age or subjects ≥18 years old. For 20 children and adolescents, aged between 5 and 22 years, diagnosed with Duchenne muscular dystrophy, a direct measurement of GFR was performed.[20] GFR was measured in these patients because current estimating GFR equations based on creatinine are particularly inaccurate in patients with such muscle disease.

2.2. "Reference standard method"

A single activity between 0.75 and 1.85 MBq of ^{51}Cr-EDTA, depending on the child's weight, in a volume of 1 mL, was injected into an antecubital vein. To ascertain intravenous administration, a 1-minute planar image of the injection site was acquired on a gamma-camera (energy window of chromium-51 (320 keV) ± 10%, LEHR collimator). To correct for rest activity, the syringe was rinsed after injection into a vial with ammoniacal water. The differential weight of this vial was determined on a high precision analytic balance. Subsequently, 9-mL blood samples were drawn from the opposite arm at approximately 15, 30, 45, 60, 120, 180, 240 and 300 minutes after the injection. Exact time of sampling was recorded. In one case (identity [ID] = 2), there was one missing concentrations at time 45 minutes. Blood samples were centrifuged at 4000 rpm for 10 minutes and 1-mL aliquots of plasma were counted in an automatic gamma counter, calibrated to the energy of chromium-51 (320 keV), together with duplicate standards and a 1-mL aliquot of the rinsing vial. Each sample was counted for 40 minutes. All values were corrected for the blanc.

The double exponential decay curve was fitted using 8 different fitting procedures (see Supplemental Material, http://links.lww.com/MD2/A842 (Section 1) for more details).

1. SI: Slope-Intercept method, with separate fitting for the late compartment (using time-points of 120, 180, 240, and 300 minutes and log-transformed concentrations) and the early compartment (using 15, 30, 45, and 60 minutes and log-transformed concentrations).
2. mSI: modified SI-method, same method as in 1, but with a common point at 120 minutes for the early (last point) and late (first point) compartment.
3. NLLS: unweighted Non-Linear Least Squares regression method based on the Levenberg-Marquardt algorithm for the full curve (non-compartmental fitting).
4. NLLS-w: same method as in 3 but using $1/Y^2$ weights (Y = concentration or counts).
5. S-NLLS: Split scenario (late vs early, no log-transformation) but based on NLLS.
6. S-NLLS-w: Split scenario as in 5, based on NLLS, using $1/Y^2$ weights.
7. mS-NLLS: modified Split scenario (using a common point at 120 minutes) but the mono-exponential decays are fitted using NLLS.
8. mS-NLLS-w: idem as 7, but with $1/Y^2$ weights.

The inclusion of the 120 minutes time-point for the early compartment may have an effect on the GFR which can be estimated from the difference between the GFR determined from the SI-method compared with the mSI method, or from comparing the results obtained with the NLLS-method and the mS-NLLS method.

For the sake of comparison, we defined a consensus result for the reference standard, by ranking the GFR-results obtained by the 8 different fitting procedures, per case, from low to high, and taking the average of the middle 4 results. This consensus result to define a "reference GFR" relies on the assumption that (at least) half of the fitting methods will return an accurate result. Averaging the middle 4 results further reduces the effect of a possible erroneous result among these 4 results.

2.3. Simplified methods

2.3.1. Based on multiple late samples. From the total concentration–time curve, we used the late samples (time ≥2 hours) to calculate the area under the slow or late component, represented by a mono-exponential decay. The accuracy of the simplified late sample procedure, combined with correction

methods for the absence of the fast or early component, namely the Bröchner-Mortensen (BM) correction formula,[17,21] and the correction formulas of Chantler,[19] Ng,[5,18] Fleming,[16] and JØdal-BM,[17] was evaluated.

2.3.2. Based on one sample. Three one-sample algorithms were applied at different late time-points to investigate the accuracy of these methods: the method proposed by Ham and Piepsz[9]; the method proposed by Jacobsson,[12] an iterative procedure that can be applied for different time-points; the method proposed by Fleming.[11]

The late sample correction formulas (Section 2) and the one-sample algorithms (Section 3) are presented in the Supplement; http://links.lww.com/MD2/A842.

2.3.3. Statistical methods

2.3.3.1. Performance statistics. To compare the GFR-results obtained from the full concentration–time curve ("reference standard GFR") with the GFR-results obtained from the simplified methods, we calculated the bias (defined as the average of the differences), the standard deviation (SD) of the differences and the root mean square error (RMSE) as the square root of the mean of the squared differences. A perfect comparison would result in bias-, SD-, and RMSE-values of 0. Lin concordance correlation coefficient (Lin CCC) combines precision and accuracy to determine how far the GFR-result obtained with the simplified method deviates from the line of perfect concordance (that is, the line at 45° on a square scatter plot) with the reference method. A perfect comparison would equal a Lin CCC-value of 1.

2.3.3.2. Simulations. Using Microsoft Excel's random number generator (RAND() provides a random number between 0 and 1), we simulated 3000 new concentration–time decays per subject, with data around the original data, by randomly generating time-points, concentrations, and injected activity deviating from the original data by no more than ±2.5%, that is, New Value = Original Value × (1 + r) with $r = [-2.5 + 5.0 \times \text{RAND}()]/100$. We fitted each of these 3000 datasets (for each subject), using the Slope-Intercept method (but discarding the negative subtractions) and calculated the GFR.

3. Results

Patient characteristics are less relevant for the current topic but are described in detail elsewhere.[20]

3.1. Reference standard method

3.1.1. Results of the fitting procedures. The fitting results are presented in Table 1. Only in 9 out of 20 cases, all 8 fitting methods were within 5% of each other (and of the consensus result).

The SI-method failed to calculate the AUC in 6 out of 20 cases, due to negative residuals (for which the logarithm could not be calculated). However, when at least 2 out of 4 time-points <120 minutes did not show negative subtractions, a fitting result could still be obtained (in 4 out of these 6 cases). In 2 cases out of 20, the difference with the consensus was between 5% and 10%, in the other 12 cases the difference was <5%.

The mSI-method failed to calculate the AUC in 1 out of 20 cases. In 15 out of 20 cases the GFR-results were within 5% of the consensus result; in 3 cases the GFR-results were within 5% to 10% and in 1 case the GFR-result was within 10% to 20% of the consensus result.

The 6 other methods, based on NLLS, were able to fit the curve in all cases, that is, to provide fit parameters from which the AUC (and thus the GFR) could be calculated. The NLLS method always provided the lowest RMSE, but reported a GFR = 0 in 1

Table 1

GFR-results (in mL/min/1.73 m^2) of the 8 fitting procedures.

ID	SI	mSI	NLLS	NLLS-w	S-NLLS	S-NLLS-w	mS-NLLS	mS-NLLS-w	Consensus
1	NA/109.6	108.8	112.2	111.0	110.0	109.9	110.3	110.9	110.6
2	160.1	159.3	160.6	160.1	159.3	160.3	159.4	160.3	160.0
3	115.3	133.1	122.6	125.0	86.6	124.4	86.6	124.4	121.7
4	156.8	146.2	158.8	156.4	158.5	157.1	158.6	157.6	157.5
5	126.5	113.6	121.8	122.9	126.2	126.8	126.2	126.5	125.5
6	159.6	156.7	160.9	161.5	160.4	160.1	160.5	160.1	160.3
7	148.6	144.1	147.1	148.7	148.1	148.2	148.1	148.2	148.2
8	NA/146.9	151.8	146.6	146.8	142.1	146.1	142.1	146.1	146.4
9	142.3	138.9	140.6	142.4	141.4	141.9	141.4	141.8	141.6
10	126.1	124.5	124.3	124.8	124.6	125.0	124.6	125.0	124.8
11	128.4	122.3	128.9	128.8	128.5	128.3	128.5	128.3	128.4
12	128.1	128.1	**0.0**[*]	113.4	129.3	127.8	130.0	129.1	128.3
13	132.8	137.3	117.7	122.7	81.5	127.2	81.5	127.2	123.7
14	225.5	222.3	225.8	227.0	225.4	225.3	225.4	225.3	225.4
15	137.2	136.3	136.6	137.3	136.9	137.2	136.9	137.2	137.0
16	NA	NA	85.6	88.7	**2.5**[*]	83.3	**2.5**[*]	83.3	85.2
17	NA/117.1	116.5	116.6	117.4	116.8	116.8	116.8	116.8	116.8
18	163.5	160.4	165.3	165.2	164.0	163.6	164.3	164.7	164.1
19	NA/126.7	131.6	127.8	127.2	126.9	126.2	126.9	126.2	127.2
20	NA	108.3	110.5	110.1	110.4	100.7	110.4	100.7	109.8

mSI = modified Slope-Intercept, mS-NLLS = modified split scenario for unweighted non-linear least squares, mS-NLLS-w = modified split scenario for weighted non-linear least squares, NLLS = non-linear least-squares, NLLS-w = weighted non-linear least-squares, SI = Slope-Intercept, S-NLLS = split scenario for unweighted non-linear least-squares, S-NLLS-w = split scenario for weighted non-linear least-squares. NA = not available due to negative subtractions, but when these were discarded, a result could sometimes be obtained.

[*] The obtained AUC was close to infinity (because the $c(t)$ curve ended [nearly] in a plateau).

case, while all other methods were relatively close to the consensus for that case. For that particular case (ID = 12), the AUC obtained from the NLLS-method tended to become infinity, because one of the B-coefficients was 0, indicating that the $c(t)$ curve ended in a plateau-value. In 19 out of 20 cases, the NLLS-method provided a GFR-result within 5% of the consensus result.

The weighted NLLS-method (NLLS-w) reported GFR-values within 5% from the consensus in 19/20 cases; only in 1 case (ID = 12) the deviation was >10%.

The S-NLLS method and mS-NLLS-method reported GFR-values within 5% of the consensus in 17/20 cases, however, in the other 3 cases (ID = 3, 13, and 16) the deviation was >20%, with 1 case (ID = 16) reporting an unrealistic GFR-value <5 mL/min.

The weighted variant of the S-NLLS method and mS-NLLS method (S-NLLS-w and mS-NLLS-w) reported GFR-values within 5% of the consensus in 19/20 cases and a difference of <10% in 1 case. No differences were observed between the fitting method and it's modification (that is, between SI and mSI, or between S-NLLS and mS-NLLS) demonstrating that using the 120 minutes time-point as a common point for the early and late compartment did not have an effect on the obtained GFR-result. Two detailed examples illustrating the effect of the fitting procedures are described in the Supplemental Material, http://links.lww.com/MD2/A842 (Section 4).

A remark should be made about patient 14 (in Table 1) who had a normal non-indexed GFR-value (for all fitting methods) of 96.6 mL/min, which was converted (×1.73/body surface area [BSA]) into an extremely high GFR-value of 225 mL/min/1.73 m^2, due to the very low BSA-value of 0.74 m^2 (height of 106 cm and weight of 19 kg).

3.1.2. Results from the simulations. Simulations were made, based on the SI-method, in which we assumed that there was error in the registration of the time-points, error in the activity and error in the registered counts (or concentrations). The summary results are shown in Table 2. In most cases, the 3000 simulations provided a GFR-result based on the SI-method when non-negative subtractions were ignored, however we also observed situations in which the simulated data could not be fitted. In Fig. 1, we present the GFR-distribution obtained after 3000 simulations for case ID = 2 and case ID = 12. The variability in obtained GFR-results is illustrated in Table 2 by the coefficient of variation (%CV), varying between 1.7% and 24.1%. It is worth noting that the simulations showing the largest %CV also showed discrepant results in the different fit methods (Table 1). Cases ID = 3, 8, 12, 13, 16, 19, and 20 show %CVs > 6% and cases 3, 12, 13, and 16 also showed fitting problems for specific fitting procedures. In case there were no fitting problems (see Table 1), the simulations (see Table 2) also had the lowest %CV (around 1.7–2.5%).

3.2. Simplified methods

3.2.1. Multiple late samples method. Fitting the late samples (all time-points ≥120 minutes) resulted in AUCs and in slow GFR-values which were all within 5% of each other, independent of the fitting method (SI, mSI, weighted or unweighted NLLS) showing the robustness of fitting the late samples with a mono-exponential decay function. As all correction formulae to calculate GFR from the slow GFR are based on indexed GFR, we indexed GFR for BSA before applying the correction formulae, in the following analyses.

We selected the S-NLLS-w method for the reference full-concentration method and also used the S-NLLS-w method to calculate the slow AUC and slow GFR from the late samples.

The area under the fast component was 16.1% ± 6.5% (range, [2.6–27.1%]) of the total AUC. Thus, the fast component does not have a large contribution in the total area. Therefore, there is a high correlation between the total area and the slow area (Fig. 2). As slow GFR (GFR_S) = Activity/Slow AUC and GFR = Activity/Total AUC, and Total AUC = 1.175 × Slow AUC (from Fig. 2), it can easily be seen that $GFR = GFR_S/1.175 = 0.85 \times$

Table 2

Statistics for GFR (in mL/min) from n simulations, based on a ±2.5% error in time-points, counts, and dosage.

ID	%CV	Mean GFR	Stdev	Min	Max	P2.5	Median	P97.5	n	GFR Ind
1	1.8%	102.5	1.8	96.9	108.0	99.0	102.5	105.9	3000	109.4
2	1.9%	110.8	2.1	97.0	116.5	106.9	110.9	114.5	3000	159.7
3	6.3%	85.6	5.4	28.6	93.8	70.4	86.7	91.1	3000	119.9
4	1.7%	108.8	1.8	104.2	113.5	105.5	108.8	112.0	3000	157.2
5	1.7%	116.2	2.0	111.1	122.5	112.6	116.2	119.8	3000	126.4
6	1.8%	100.5	1.8	75.5	104.9	97.3	100.5	103.6	3000	159.2
7	1.7%	113.3	1.9	108.4	119.0	109.8	113.3	116.8	3000	148.1
8	6.4%	105.6	6.8	5.4	114.5	89.1	106.9	112.1	3000	144.9
9	1.8%	105.9	1.9	99.3	111.7	102.4	105.9	109.3	3000	142.3
10	2.3%	109.9	2.6	64.1	116.4	105.7	110.0	114.1	3000	125.8
11	1.7%	129.9	2.2	123.5	136.0	125.9	129.9	133.9	3000	128.2
12	24.1%	113.6	27.3	−671.9*	1004.4	106.0	114.1	119.4	3000	126.4
13	6.9%	87.2	6.0	21.5	94.5	71.7	88.6	92.5	3000	129.8
14	1.7%	96.4	1.6	91.7	100.6	93.5	96.4	99.3	3000	225.9
15	1.7%	118.9	2.1	113.6	124.4	115.2	118.9	122.7	3000	136.9
16	7.1%	84.2	6.0	70.2	88.9	72.4	85.4	88.8	8	84.4
17	2.6%	102.4	2.6	65.1	108.5	97.2	102.6	106.1	2998	116.1
18	1.7%	75.6	1.3	71.8	79.6	73.2	75.6	78.0	3000	163.1
19	8.3%	90.9	7.6	6.8	100.7	71.0	92.8	97.8	3000	121.6
20	9.4%	75.5	7.1	6.2	83.0	55.5	77.1	81.1	1469	106.4

GFR Ind = mean GFR × 1.73/BSA (mL/min/1.73 m^2).

* A negative value may appear when one of the fitted power coefficients B is negative; %CV = coefficient of variation; P2.5 and P97.5 are the 2.5th and 97.5th percentile of the simulated results.

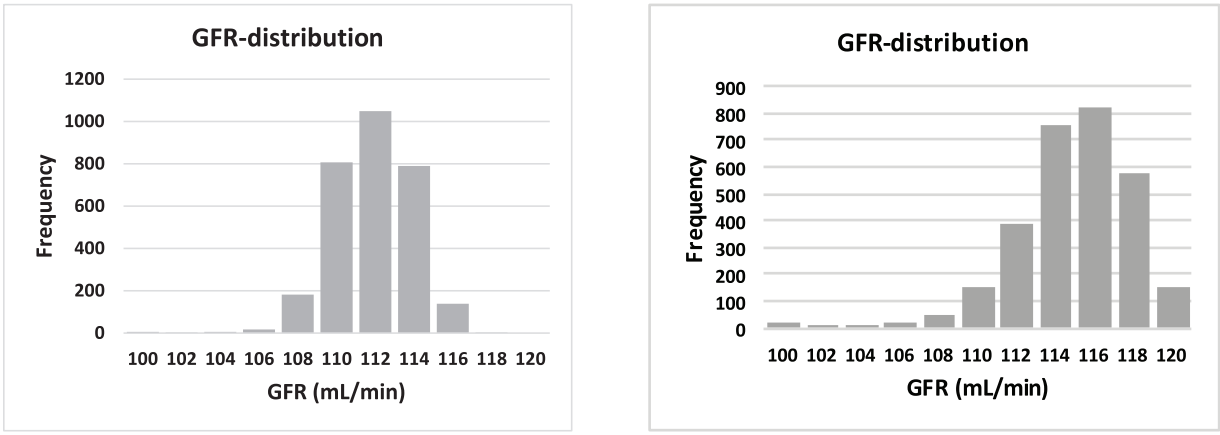

Figure 1. GFR distributions for the 3000 simulations of cases ID = 2 (left) and ID = 12 (right). GFR = glomerular filtration rate.

GFR_S, a result that is very close to the Chantler correction formula[20] of $GFR = 0.87 \times GFR_S$.

The final GFR can be obtained from the slow GFR combined with a correction formula to compensate for the absence of the fast component. In Fig. 3, the GFR indexed for BSA obtained from the full compartment model is plotted against the slow GFR, together with some curves obtained from the correction formulas. In Table 3, the performance statistics for the late sample models as compared with the "reference standard" GFR are presented.

The correction formulas of Ng, Fleming, and JØdal-BM are all based on $GFR = GFR_S/(1 + f \times GFR_S)$. Therefore, $f = (GFR_S - GFR)/(GFR_S \times GFR)$ can be calculated for each child. The mean value of f was 0.0012 with SD = 0.0004, matching Ng's f-value. BM's correction formula can be applied before or after BSA-indexing (in Table 3, BM_BSA is first BM correction, then BSA-indexing). In the case of children with $BSA < 1.73\,m^2$, indexing increases the GFR and shifts the indexed slow GFR towards the region where the non-linearity in the BM correction equation becomes more important. This may explain why correcting before indexing gives less biased results compared with indexing before correcting.

3.2.2. One-sample methods. Using the S-NLLS-w GFR as the reference, the performance of the one-sample methods for different time-points is given in Table 3. Jacobsson iterative method at time = 120 minutes gives the best results in terms of lowest RMSE and highest Lin CCC.

3.2.3. Simulation results. Using the late samples (or the single sample at 120 minutes only) we also performed 3000 simulations (see Supplemental Material, http://links.lww.com/MD2/A842 section 5), and found that %CVs (defined as the SD of the GFR obtained from 3000 simulations divided by the mean GFR of the 3000 simulations) were always smaller than 4%, also for the cases that gave large %CVs when the full decay curve was fitted in the simulations, indicating that the variability in the problematic fittings are due to the fast or early component. The Chantler-equation gave the highest %CV which were always between 2.0% and 4.0%. All other correction formulas had % CVs < 3.0%. The mean bias was calculated for the 3000 simulations per subject, and the overall mean bias of the 20 children was very similar to the reported bias in Table 3. The results obtained with the Ng-correction formula gave the lowest overall mean bias. In Table 4 Lin CCC and Pearson correlation

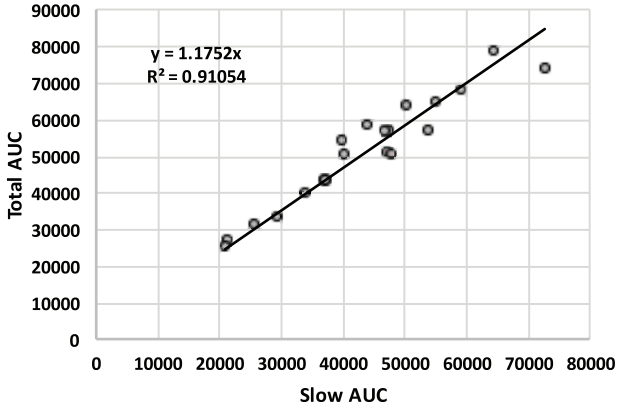

Figure 2. Relationship between slow and total AUC. AUC = area under the curve.

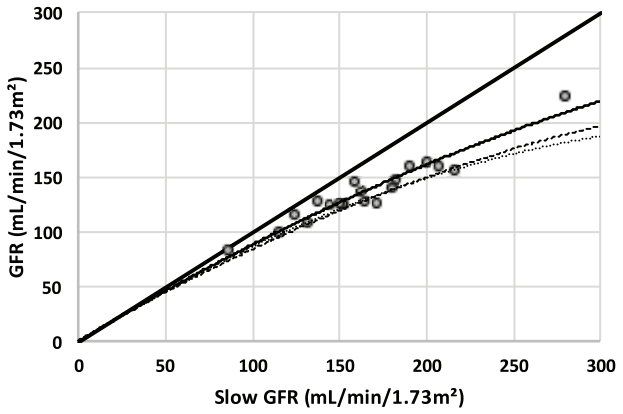

Figure 3. GFR (indexed for BSA) obtained from S-NLLS-w against slow GFR (indexed for BSA) obtained from the slow component of the S-NLLS-w method. Diagonal is the identity line. The solid curve is the Ng-correction formula ($f = 0.0012$); the dashed curve is the Fleming correction ($f = 0.0017$) and the dotted curve is the BM-correction. BSA = body surface area, GFR = glomerular filtration rate, S-NLLS-w = split scenario for weighted non-linear least-squares.

Table 3

Performance statistics of the different multiple late-samples and one-sample methods.

	Method	RMSE	Lin CCC	Bias	SD
Multiple late samples methods	BM	13.3	0.875	8.5	10.5
	Ng	7.9	0.960	0.1	8.2
	Fleming	12.7	0.891	9.2	9.0
	Jodal-BM	14.8	0.844	9.9	11.3
	Chantler	12.6	0.925	−6.8	10.9
	BM_BSA	8.4	0.964	−1.7	8.4
	Time, min	**RMSE**	**Lin CCC**	**Bias**	**SD**
Jacobsson iterative one sample method	120	8.2	0.961	2.5	8.0
	180	11.6	0.915	−2.1	11.7
	240	15.9	0.837	−2.3	16.2
	300	19.3	0.749	−0.7	19.8
	Time, min	**RMSE**	**Lin CCC**	**Bias**	**SD**
Adjusted Jacobsson one sample method	120	11.6	0.922	5.0	10.7
	180	16.7	0.813	−4.1	16.6
	240	22.5	0.661	−6.8	22.0
	300	26.8	0.507	−7.0	26.5
	Time, min	**RMSE**	**Lin CCC**	**Bias**	**SD**
Piepsz	120	12.2	0.933	−4.2	11.8
	Time, min	**RMSE**	**Lin CCC**	**Bias**	**SD**
Fleming	120	21.5	0.686	17.4	12.9
	180	22.0	0.657	17.0	14.4

BM_BSA = correcting with BM before indexing for BSA; Time (min) after injection; Adjusted Jacobsson 1-point method uses Vs = 246 × Wt in the iteration algorithm; RMSE = root mean square error; Lin CCC = Lin concordance correlation coefficient; SD = standard deviation.

coefficients (linear correlation coefficient) between the different methods are presented for the GFR-results of the 20 subjects. Because the results are mostly in the very high GFR region, non-linearity between the GFR and the slow GFR (GFR_S) is most present, which is reflected in the poorest correlation coefficients between BM (a quadratic correction method) and Chantler (a linear correction method). As also Piepsz' method is a linear method, there is also poor correlation with BM. The Ng correction formula deviates the least from the linear line, which is reflected in the high correlation between Ng and Chantler, and between Ng and Piepsz. Note that among the late sample methods, Ng also shows the highest correlation with the GFR obtained from the full decay curve. BM, Fleming, and BMJ show the highest correlations among themselves. The one-sample methods at 120 minutes also show high correlations with GFR obtained from the full concentration–time curve, but also with the late sample methods, especially Jacobsson iterative method.

4. Discussion

In the current study, we measured the full plasma disappearance curve after bolus injection of ^{51}Cr-EDTA in 20 Duchenne muscular dystrophy patients. Most of these children have BSA < 1.73 m^2 and the indexed GFR is mostly high. Because we experienced problems when fitting the 2 compartment curve with the slope-intercept method (6 out of 20 curves could not be analyzed with the SI-method), we evaluated 8 different fitting procedures. Our analysis demonstrates the flaws of each fitting procedure. Goodness of fit as measured by the sum of squares of residuals will always result in the best fit for the NLLS full

Table 4

Lin concordance correlation coefficient (upper triangle) and Pearson correlation coefficients (lower triangle) calculated from the mean GFR-values obtained from the 3000 simulations and calculated with the SI-method from the full compartment model (GFR), from the late sample methods (Bröchner-Mortensen [BM], Ng, Fleming [Flem], Bröchner-Mortensen-Jodal [BMJ], Chantler [C]), and from the one-sample methods (Piepsz and Jacobsson at 120 minutes [Jac120]).

	GFR	BM	Ng	Flem	BMJ	C	Piepsz	Jac120
GFR		0.869	0.958	0.886	0.838	0.922	0.923	0.958
BM	0.941		0.928	0.996	0.996	0.796	0.765	0.908
Ng	0.958	0.997		0.935	0.902	0.947	0.910	0.968
Flem	0.954	0.998	1.000		0.992	0.817	0.791	0.920
BMJ	0.931	0.998	0.994	0.996		0.767	0.736	0.878
C	0.967	0.989	0.998	0.996	0.986		0.970	0.924
Piepsz	0.974	0.950	0.965	0.962	0.944	0.972		0.931
Jac120	0.962	0.968	0.973	0.972	0.957	0.971	0.980	

GFR = glomerular filtration rate.

compartment model, but this does not guarantee the accuracy of the reported GFR. The slope-intercept method fails to fit the curve in (too) many occasions. The weighted NLLS, weighted S-NLLS, and weighted mS-NLLS seemed to be the most robust fitting procedures for the data at hand, but also these methods failed in some occasions to report a reliable GFR-result. This raises the question of how to perform quality control that guarantees that the reported GFR is the most accurate result. We have shown that it may not be good practice to simply switch from the SI-method (when the fit fails) to the NLLS-method, as the reported GFR may also not be accurate. In fact, our simulations have shown that when the SI-method fails, there might be a more general problem with the data at hand. At the same time, these simulations offer a quality control check (in terms of the %CV) and allow to calculate the best GFR-result as the mean or median of the 3000 simulations. In case of failure of the SI-method, small changes in the data (here realized by introducing a small error in time, concentration, and dose of ±2.5%) would mostly lead to successful fits and thus to valid results. The 3000 simulations result in a distribution of GFR-values that are "peaking" at the most reliable value for mGFR.

As all fitting procedures have problems to fit the early compartment, a good alternative is to use late samples only (≥2 hours) and apply the slope-intercept method for the slow component, which results in the so-called "slow" GFR (GFR_S). This slow GFR then needs to be corrected for the absence of the early compartment, but the contribution of the AUC from the early compartment is logically much smaller than the contribution of the AUC from the late compartment. Consequently, the combination of determining the slow GFR with a correction formula (the preferred correction formula in children for the data at hand is the Ng correction formula) is a more robust method (more robust in the sense that the reported GFR is reliable) than the so-called reference standard method, where GFR is obtained from the full concentration–time curve.

Measuring GFR in children is not straightforward and not many studies have reported GFR via plasma disappearance in children. Schwartz compiled data for GFR determined by inulin clearance in normal children and young adults and reported GFR-values of 110–115 mL/min/1.73 m^2.[4] Piepsz et al[6,7] carried out a study in 623 apparently healthy children, aged 0.1 to 15 years, using Piepsz' one-sample method (at 120 minutes) based on ^{51}Cr-EDTA, and this is—to our knowledge—the only study in healthy children reporting directly measured GFR. Pottel et al[22,23] used these data to fit GFR against age and reported that GFR in healthy children aged >2 years becomes stable around the value of 107.3 mL/min/1.73 m^2. Schwartz[24] has criticized the possible uncertainty of the one-sample method which could result in substantial variability in the determined GFR. However, we have shown in this study that the one-sample method may be more reliable than the full concentration–time method. Tøndel et al[25] performed a study in 96 children with chronic kidney disease (median GFR of 66 mL/min/1.73 m^2), median age of 9.2 years (range, 3 months–17.5 years) using plasma iohexol clearance at 7 time-points within 5 hours as the reference method. The aim of their study was to evaluate the performance of different single-time point formulas. They recommended the Fleming method at 3 hours, which we cannot confirm in our analysis, probably because of the high GFR in our study (with median GFR of 130 mL/min/1.73 m^2). Tøndel calculated the GFR using the slope-intercept method for the two-compartment model, and they reported that for 3 patients the two-compartment slope-intercept method could not be used due to negative subtractions after removing the slow component from the curve. For these patients, they fitted the two-compartment model using non-linear least squares regression (NLLS). We here showed that this strategy is not without danger without quality control. Schwartz performed a pilot study in 27 children and 2 adults with various kidney diseases, median age of 14 years (IQR: 12–18), using iohexol as the exogenous marker, with multiple early and late time-points. They used the slope-intercept method for the two-compartment model, but did not report fitting problems.[3] We believe that fitting problems are commonly not reported as it is easy to switch to another fitting procedure. However, there is no guarantee that the alternative fitting procedure, although it is reporting a GFR-result, is giving an accurate result. All fitting procedures have shortcomings due to the early component. Fitting problems point to a lack of quality in the early component of the concentration–time decay curve. Therefore, late sample methods, combined with correction equations to account for the missing early compartment, seem to be more robust than fitting the complete concentration–time curve.

Limitations of this study are the low sample size, no subjects with low GFR and only male white children with a specific disease. However, because fitting errors are due to the first compartment there is no theoretical reason to believe that it may be different in healthy children or in children with CKD. Moreover, it could be worse in severe CKD because it may be expected that the concentration–time decays to a plateau value, resulting in an AUC going to infinity (and thus subject to large error). Ideally, the number of samples in the early phase should be higher to optimize the fitting quality. Only by multiple early sampling, it would be possible to truly decipher the nature of the early component, but this is very difficult from a practical point of view, especially in children. Others[3,25] used an equivalent and even a smaller number of early samples. Most of our patients had normal to high GFR-values and our results need to be confirmed in children with CKD. However, from a similar analysis in older adults, we have shown that the same type of fitting problems occurred in both the low and high GFR-range. Absence of low GFR in the current study may also be problematic to make conclusions about the single sample method.

In conclusion, we can say that the simplified method determining the slow GFR combined with the Ng correction formula to estimate the early compartment shows acceptable accuracy, compared with the GFR obtained from the full compartment decay curve. In our patients in the high GFR range, the one-sample iterative method of Jacobsson, using the 120 minutes time-point, also gives acceptable results, comparable to the multiple late-samples method combined with the Ng-correction. Also the very simple one-sample method of Ham and Piepsz shows reasonable accuracy. Other one-sample methods (Fleming, and Jacobsson iterative method using $Vs = 246 \times Wt$) were not as good. The one-sample methods may partially solve the discomfort of the full concentration–time methods which require multiple early and late samples. However, there is no way for a quality control, which is only possible when ≥3 late samples are used.

Author contributions

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