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ORIGINAL ARTICLE

Single- versus multiple-sample method to measure glomerular filtration rate

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ABSTRACT

Background. There are many different ways to measure glomerular filtration rate (GFR) using various exogenous filtration markers, each having their own strengths and limitations. However, not only the marker, but also the methodology may vary in many ways, including the use of urinary or plasma clearance, and, in the case of plasma clearance, the number of time points used to calculate the area under the concentration–time curve, ranging from only one (Jacobsson method) to eight (or more) blood samples.

Methods. We collected the results obtained from 5106 plasma clearances (iohexol or ^{51}Cr-ethylenediaminetetraacetic acid (EDTA)) using three to four time points, allowing GFR calculation using the slope–intercept method and the Bröchner-Mortensen correction. For each time point, the Jacobsson formula was applied to obtain the single-sample GFR. We used Bland–Altman plots to determine the accuracy of the Jacobsson method at each time point.

Results. The single-sample method showed within 10% concordances with the multiple-sample method of 66.4%, 83.6%, 91.4% and 96.0% at the time points 120, 180, 240 and ≥300 min, respectively. Concordance was poorer at lower GFR levels, and this trend is in parallel with increasing age. Results were similar in males and females. Some discordance was found in the obese subjects.

Conclusion. Single-sample GFR is highly concordant with a multiple-sample strategy, except in the low GFR range (<30 mL/min).

Keywords: biomarkers, CKD, ^{51}Cr-EDTA, glomerular filtration rate, iohexol

INTRODUCTION

Measuring glomerular filtration rate (GFR) is still considered the best way to assess renal function [1–3]. Because estimated GFR (eGFR) is influenced by so-called non-GFR determinants [4, 5], measured GFR (mGFR) has an important place in specific patients and/or clinical scenarios [1–3]. There are different ways to measure GFR. Indeed, various tracers and methods are available, each having their own strengths and limitations [3, 6, 7]. Besides, the methodology to measure GFR, namely urinary versus plasma clearance, can also influence the results of mGFR. Briefly, urinary clearance remains the reference method, especially in the context of abnormal extracellular volume (ascites or oedema) [2, 8–10]. However, repeated and timed urine collection is particularly challenging, error prone and sometimes impossible, especially in young children or older patients [11]. Plasma clearance is thus frequently preferred and the concordance with urinary clearance is good [8, 9, 11–14]. However, methodologies to measure GFR by plasma clearance also differ with regards to the number and timing of samples [8, 13, 15–19]. In the present analysis, we compared the results of mGFR obtained by the two most widely used plasma clearance methods: the multiple-sample method, originally described by Bröchner-Mortensen [20] and the single-sample method as described by Jacobsson [21].

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MATERIALS AND METHODS

Participants

We collected data on plasma clearances for 5344 subjects in four different centres in Europe: Lyon, France ($n = 2800$), Paris, France ($n = 1862$), Berlin, Germany ($n = 570$) and Liège, Belgium ($n = 112$). All centres are experts in GFR measurement and have previously published their data in whole or in part related to different topics [22–25]. Research was thus approved by the respective ethics committees. Data from Paris, Lyon and Liège were obtained from patients referred for mGFR for clinical purposes. Data from Berlin were obtained in subjects aged 70 years and older in the framework of the Berlin Initiative Study (BIS).

Description of GFR measurements

All measurements were performed in the morning in fasting (or after a light protein-free breakfast) and resting conditions. These 5344 results share the same characteristics: all subjects were adults, there was one single GFR result per subject and there were at least three plasma samples available at three different time points (at ~120, 180 and 240 min after injection). The exogenous marker used was iohexol in Liège, Berlin and Lyon, and ^{51}Cr-ethylenediaminetetraacetic acid (EDTA) in Paris. Among the 5344 clearance results, 715 were obtained with one additional sample obtained at a later time point (i.e. a fourth time point at 300 min or later after injection). The slope–intercept method described by Bröchner-Mortensen [20] was used to calculate mGFR with three or four available samples and is hereafter referred to as the multiple-sample method. Goodness of fit was judged by the R^2-value and only those fits with $R^2 \geq 0.975$ ($n = 5106$ overall, and $n = 657$ with four time points) were retained for further analysis.

The 'slow' GFR was calculated as the dose or quantity of iohexol or ^{51}Cr-EDTA injected, divided by the area under the concentration–time curve, calculated from the slope and intercept results. As this 'slow' GFR was obtained from the slow compartment model only, the correction proposed by Bröchner-Mortensen was applied to compensate for the absence of the 'fast' first decay component, which corresponds with the mixing of the marker in its volume of distribution (V_d): GFR (mL/min) $= 0.990778 \times$ 'Slow' GFR $- 0.001218 \times$ ('Slow' GFR)^2.

The single-sample method described by Jacobsson was also used to calculate GFR and is hereafter referred to as the single-sample method [21]. Single-sample mGFR was calculated for each time point, leading to three to four results that were compared to the multiple-sample result. The so-called iterative method was used as single-sample method (see Supplementary Data).

Body surface area (BSA) indexation of GFR is controversial [26]. Moreover, there is also controversy as to when to apply the BSA indexation (before or after the Bröchner-Mortensen correction) [27]. Therefore, we decided to present all mGFR results non-indexed for BSA.

Strategies based on estimating GFR and subgroup analyses

The choice of the best time point for the single-sample method remains an approximation (see Supplementary Data for the optimal timing according to Jacobsson). For the single-sample method, which is routinely performed in Sweden, it is recommended to adapt the time point of the measurement based on the expected GFR calculated from creatinine-derived estimating equations. A sample at 180 min is recommended if the eGFR is >50 mL/min/1.73 m^2 and a sample at 300 min is recommended if the eGFR is <50 mL/min/1.73 m^2 [16]. We evaluated this strategy and compared it either with an alternative time point strategy, namely using another time point (at 240 min if the eGFR was >50 mL/min/1.73 m^2 and at 300 min or later if the eGFR was <50 mL/min/1.73 m^2) or with a strategy using the same fixed recommended time point in all cases. Among the 657 patients with a $\geq$300 min time point, serum creatinine sampled at the same day of GFR measurement was available for 639 patients. All the creatinine results were traceable to isotope dilution mass spectrometry [28]. The Full Age Spectrum (FAS) equation based on creatinine was used to estimate GFR (GFR_{FAS}) [29].

Finally, analyses were performed in subgroups according to GFR levels [defined based on the multiple-sample method in six categories: >130,]90–130],]60–90],]45–60],]30–45], $\leq$30 mL/min), gender, body mass index (BMI) ($<$18.5, [18.5–25[, [25–30[, [30–35[, [35–40[and $\geq$40 kg/m^2, respectively) and age (categorized in decades).

Statistics

Distribution of mGFR values was normal and data were expressed as mean $\pm$ standard deviation (SD). We defined the multiple-sample method as the reference method. We then calculated Lin's concordance correlation coefficient [30] between the multiple-sample method and the single-sample results at different time points. The bias (systematic difference) between the methods was also calculated as the mean difference between single- and multiple-sample results. The precision (random error) was calculated as the SD of the bias. The relative difference between the two methods was calculated as the bias divided by the multiple-sample result and expressed as a percentage. Then, we considered the concordance (as the percentage of relative difference) within 10% and 5% of the multiple-sample result. Concordances between single- and multiple-sample methods were compared by Exact McNemar tests.

RESULTS

Among the 5106 study participants, mean age was 54 ± 17 years and 42.6% were women. Mean height and body weight were 168 ± 10 cm and 73 ± 17 kg, respectively. Mean BMI and BSA were 26 ± 6 kg/m^2 and 1.85 ± 0.24 m^2, respectively. Table 1 summarized data in the whole cohort and according to centres. For all patients, three different time points were available: 120 min (mean 126 ± 7 min, range: 110–150 min), 180 min (mean 186 ± 7 min, range: 165–210 min) and 240 min (mean 245 ± 8 min, range: 201–279 min). In 657 subjects, a fourth time point was available after at least 260 min

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Table 1. Characteristics of population

	All ($n = 5106$)	Liège ($n = 110$)	Berlin ($n = 517$)	Paris ($n = 1796$)	Lyon ($n = 2683$)
Age (years)	54 ± 17	53 ± 13	78 ± 6	50 ± 14	52 ± 16
Gender (% of women)	42.6	47.3	43.5	41.2	43.2
Weight (kg)	73 ± 17	78 ± 20	77 ± 14	73 ± 16	72 ± 17
Height (cm)	168 ± 10	170 ± 11	166 ± 8	168 ± 10	167 ± 10
BMI (kg/m^2)	26 ± 6	27 ± 6	28 ± 4	26 ± 5	26 ± 6
BSA (m^2)	1.85 ± 0.24	1.93 ± 0.28	1.90 ± 0.20	1.86 ± 0.24	1.83 ± 0.25
GFR: multiple sample (three points) (mL/min)	65 ± 26	84 ± 29	65 ± 18	66 ± 27	64 ± 26
GFR: single sample (at 240 min) (mL/min)	66 ± 26	82 ± 26	65 ± 17	66 ± 26	63 ± 24

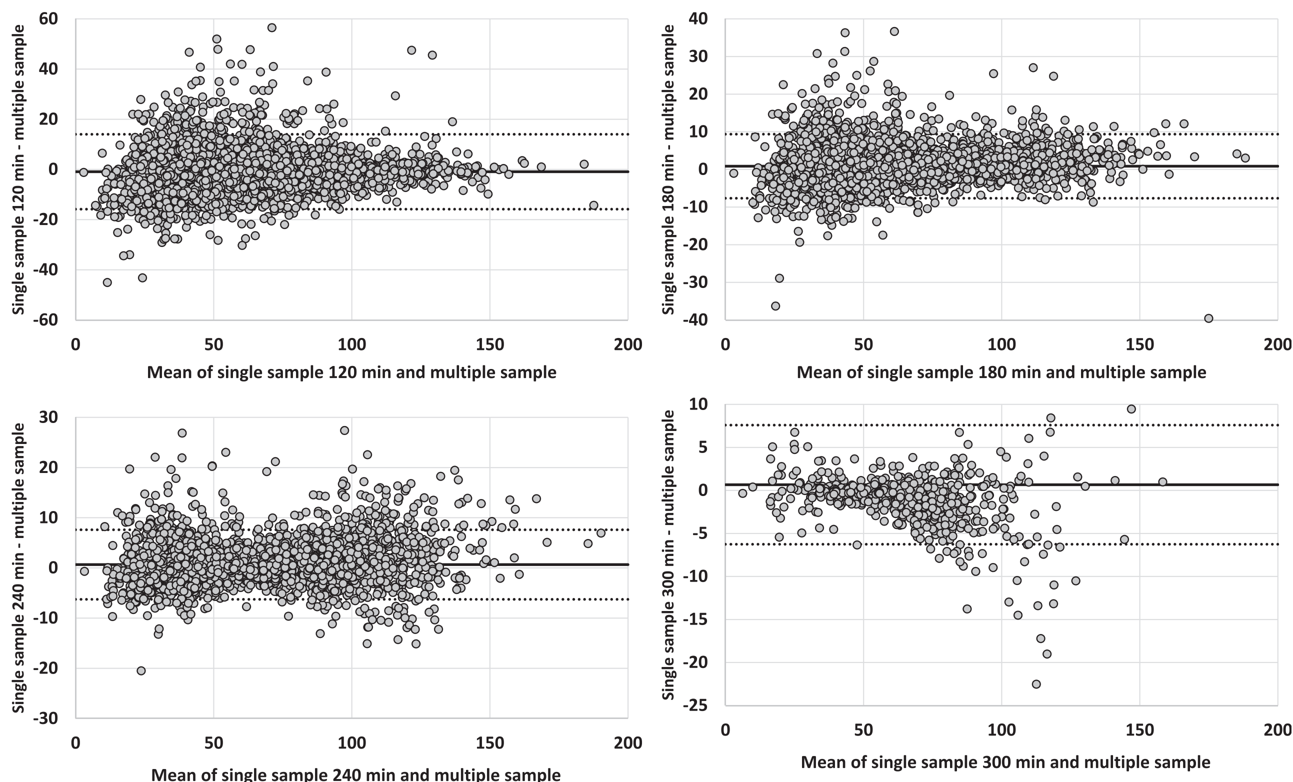

FIGURE 1: Bland-Altman plots for the multiple sample versus single sample methods at different time-points.

(hereafter called 'GFR at 300 min') (mean: 307 ± 93 min, range: 261–2360 min).

Mean mGFR in the 5106 subjects with the multiple-sample technique was 65.2 ± 25.7 mL/min. Mean mGFR with the single-sample technique at 120, 180, 240 and 300 min was 64.2 ± 26.3 ($n = 5106$), 66.0 ± 26.4 ($n = 5106$), 65.8 ± 26.3 ($n = 5106$) and 65.5 ± 22.0 mL/min ($n = 657$), respectively. Lin's concordance correlation coefficient between multiple- and single-sample GFR at 120, 180, 240 and 300 min was 0.9563 [95% confidence interval (CI) 0.9539–0.9586], 0.9856 (0.9848–0.9863), 0.9904 (0.9899–0.9909) and 0.9898 (0.9883–0.9912), respectively.

The biases between mGFR by multiple sample versus single sample at 120, 180, 240 and 300 min were −0.9 ± 7.6 ($n = 5106$), +0.9 ± 4.3 ($n = 5106$), +0.7 ± 3.5 ($n = 5106$) and −1.2 ± 3.0 mL/min ($n = 657$), respectively. A Bland–Altman plot of both the absolute and relative difference versus average is presented in Figures 1 and 2, respectively. The relative difference in Figure 2 can also be interpreted as the concordance. The concordance within 10% between multiple sample and single sample at 120, 180, 240 and 300 min was 66.4%, 83.6%, 91.4% and 96.0%, respectively. The concordance within 5% was 44.7%, 67.3%, 77.1% and 82.2%, respectively. All these concordances were significantly different from each other ($P < 0.0001$). There was a better concordance between the multiple- and the single-sample method when the concentration at 240 min ($n = 5106$) or at 300 min ($n = 657$) was considered compared with the concentration at 120 min or 180 min ($P < 0.0001$).

Subanalysis of the cohort with four samples available ($n = 657$)

The mean GFR by the multiple-sample method in these 657 patients was 66.7 ± 23.0 mL/min, which was not different from the mean GFR in the total cohort. The bias between mGFR by multiple sample versus the single sample at 120, 180, 240 and 300 min was −0.4 ± 7.0, +1.7 ± 3.6, +0.6 ± 2.6 and −1.2 ± 3.0 mL/min, respectively. The concordance within 10%

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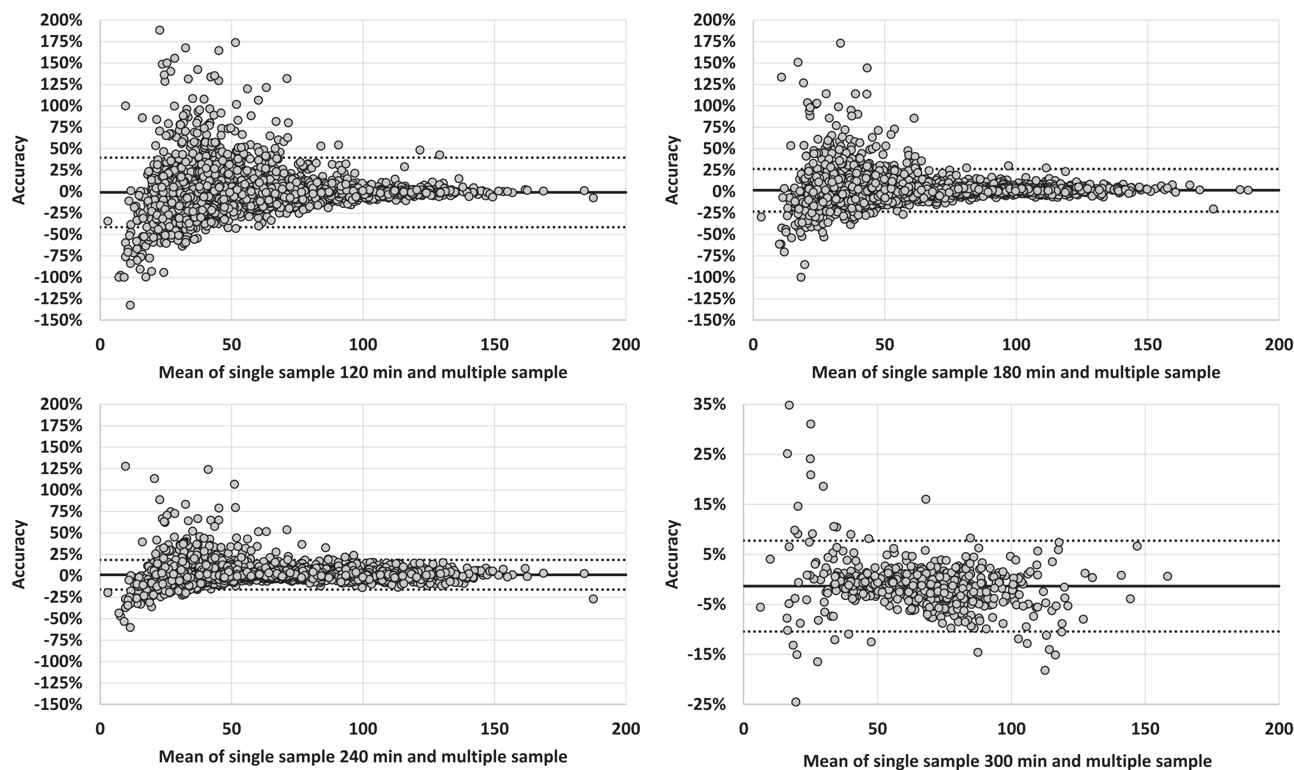

FIGURE 2: Bland-Altman plots (relative difference or accuracy) for the multiple sample versus single sample methods at different time-points.

between multiple sample and single sample at 120, 180, 240 and 300 min was 69.7%, 86.3%, 93.9% and 96.0%, respectively. The concordance within 5% was 44.3%, 66.7%, 82.3% and 82.2%, respectively. The results showed a better concordance between the multiple- and the single-sample method when the concentration at 240 min or 300 min was considered ($P < 0.0001$). The concordance result at 240 min was compared with the strategy of a modified timing according to the eGFR by FAS in 639 adults with serum creatinine available. The Swedish strategy gave a concordance within 5% and 10% of 77.6% and 93.7%, respectively. Concordance within 10% with multiple-sample using the Swedish recommendation (93.7%) was not better than using the 240 min time point (93.9%), whereas concordance within 5% was worse (77.6% versus 83.2%, $P = 0.0004$) than using the fixed time point at 240 min. Defining our own strategy (by considering the concentration at 240 min if GFR_{FAS} was >50 mL/min/1.73 m^2 and at 300 min if GFR_{FAS} was <50 mL/min/1.73 m^2), we observed a concordance within 5% and 10% of 88.7% and 96.6%, respectively. Both concordances were better than concordances with a fixed time point at 240 min ($P < 0.0001$).

Comparison of concordance within 10% according to GFR levels (Tables 2 and 3)

Tables 2 and 3 illustrate the worse concordance between multiple- and single-sample methods in the low GFR ranges. In these low ranges, concordance was better when the single concentration at the latest time point was considered, but the concordance within 10% remained low for GFR <30 mL/min (44.1% for the single sample at 240 min in the total cohort and 65.6% at 300 min in the 657 subjects-cohort with four samples).

Table 2. Comparison of concordance within 10% between the multiple-sample and the single-sample method (at different time points) according to GFR levels ($n = 5106$)

GFR range (mL/min)	120 min (%)	180 min (%)	240 min (%)
≤30 ($n = 313$)	20.8	29.4	44.1
]30–45] ($n = 889$)	34.5	59.1	83.6
]45–60] ($n = 1205$)	56.5	85.5	96.9
]60–90] ($n = 1828$)	81.9	96.4	98.2
]90–130] ($n = 813$)	96.3	98.4	94.3
>130 ($n = 58$)	100	98.3	94.8

In the total cohort, the single sample at 240 min was better than other time points for GFR < 90 mL/min, and only slightly worse for GFR ≥ 90 mL/min where the time point of 180 min resulted in better accuracy. In the low GFR range (<30 mL/min), the single-sample method systematically yielded higher results than the multiple-sample method, except for the 300 min time point.

Comparison of concordance within 10% according to gender

There was no difference in concordance according to gender in our cohort.

Comparison of concordance within 10% according to BMI

In Table 4, concordance within 10% is shown according to the BMI category in the total cohort. Concordance within 10% was slightly lower in underweight as compared with normal weight subjects. The differences were, however, much more

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Table 3. Comparison of concordance within 10% between the multiple-sample and the single-sample method (at different time points) according to GFR levels in subjects with four samples ($n = 657$ or $n = 639$ for those with serum creatinine available)

GFR range (mL/min)	120 min (%)	180 min (%)	240 min (%)	300 min (%)	Swedish strategy ($n = 639$)	Our strategy ($n = 639$)
≤30 ($n = 32$)	15.6	25.0	37.5	65.6	64.5 ($n = 31$)	64.5 ($n = 31$)
]30–45] ($n = 82$)	41.5	62.2	82.9	93.9	93.7 ($n = 79$)	91.1 ($n = 79$)
]45–60] ($n = 137$)	56.2	87.6	97.1	99.3	89.5 ($n = 133$)	98.5 ($n = 133$)
]60–90] ($n = 323$)	81.7	94.4	99.7	99.7	94.6 ($n = 318$)	99.7 ($n = 318$)
]90–130] ($n = 77$)	93.5	93.5	98.7	89.6	100 ($n = 74$)	98.7 ($n = 74$)
>130 ($n = 6$)	100	100	100	100	100 ($n = 4$)	100 ($n = 4$)

Table 4. Comparison of concordance within 10% between the multiple-sample and the single-sample method (at different time points) according to BMI ($n = 5106$)

BMI (kg/m^2)	120 min (%)	180 min (%)	240 min (%)	Mean mGFR (multiple sample) (mL/min)
≤18.5 ($n = 287$)	67.9	80.5	87.8	56.7
]18.5–25] ($n = 2221$)	71.2	86.5	92.8	63.5
]25–30] ($n = 1600$)	65.9	83.9	92.6	67.0
]30–35] ($n = 692$)	59.5	80.2	88.3	68.7
]35–40[($n = 198$)	52.0	74.2	87.9	67.9
≥40 ($n = 108$)	42.6	63.9	79.6	67.3

Table 5. Comparison of concordance within 10% between the multiple-sample and the single-sample method (at different timings) according to age ($n = 5106$)

Age (years)	120 min (%)	180 min (%)	240 min (%)	Mean mGFR (multiple sample) (mL/min)
<20 ($n = 85$)	80.0	97.6	96.5	78.0
[20–30[($n = 389$)	76.3	91.3	91.0	73.6
[30–40[($n = 639$)	75.1	88.7	93.6	75.7
[40–50[($n = 880$)	71.0	87.3	93.3	71.9
[50–60[($n = 1133$)	67.3	83.8	91.8	65.5
[60–70[($n = 961$)	58.7	78.1	89.2	56.8
[70–80[($n = 729$)	61.5	81.8	90.7	58.6
[80–90[($n = 255$)	51.4	69.0	89.0	50.6
≥90 ($n = 35$)	48.6	60.0	77.1	44.0

prominent in the highest BMI categories. Differences of concordances in high BMI categories were not influenced by the level of GFR. There was no systematic difference between single- and multiple-sample results according to BMI. In the cohort of 657 patients with four samples available, the same trends were observed.

Comparison of concordance within 10% according to age

In Table 5, concordance within 10% was shown according to age category in the whole cohort. Concordance within 10% was better in young adults at all time points and poorer in older adults, compared with the concordance within 10% in the whole cohort ($n = 5106$). This was particularly relevant in subjects older than 60 years for the single-sample method at 120 and 180 min and in subjects older than 90 years for the single-sample method at 240 min. The difference in concordance between older adults and others was lower with the single sample at 240 min in comparison with 120 and 180 min. However, it should be highlighted that mean mGFR (by multiple-sample) also decreased according to age (see results according to GFR levels). A two-way ANOVA was performed on relative differences on concordances at 240 min. Age category was not significant, but GFR classification was highly significant as the interaction term ($P < 0.0001$), suggesting that difference in GFR, not age, was the main driver of differences between single- and multiple-results.

We observed a positive bias (overestimation by the single-sample method) that became negative (underestimation by the single-sample method) with aging. The bias was null at the age of 33, 34 and 48 years for the single-method at 120, 180 and 240 min, respectively.

Comparison between centres

The concordances within 10% between the multiple- and the single-sample methods were similar in all centres. This was particularly illustrated by the comparison between the multiple-sample method and the single-sample method at 240 min: 95.4% in Berlin ($n = 517$), 93.6% in Liège ($n = 110$), 91.9% in Lyon ($n = 2683$) and 90.5% in Paris ($n = 1796$) with an overall concordance of 91.4%. Results were similar when iohexol and ^{51}Cr-EDTA were analysed separately. At time 240 min, the observed mean relative difference with multiple-sample was

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very similar (−1.5% versus −1.0% for ^{51}Cr-EDTA and iohexol, respectively).

DISCUSSION

In the present study, we showed that the performance of the single-sample plasma clearance method was similar to the more complex multiple-sample plasma clearance. Discrepancies were, however, observed in some specific clinical settings, especially in patients with very high BMI (≥40 kg/m^2) and in the low GFR range (GFR <30 mL/min). The single-sample method has the advantage of being less cumbersome and less costly than the multiple-sample clearance. This simplified method may thus be considered, as recently suggested by a European panel of experts, in clinical practice and also in clinical research, especially for large epidemiological trials [2, 8]. Except in specific subgroups, a concordance within 10% and 5% as high as 95% and 80% was found between single- and multiple-sample methods in case the adequate time point was chosen. It is important to underline these results of high concordance as we used a very stringent criterion (within 10% and even 5%) to define concordance. Indeed, in the comparison between mGFR and GFR estimating equations, a concordance within 30% is usually considered [31–33].

According to different patient characteristics available in the current analysis, both BMI category and GFR levels impacted the concordance between the two methods. Although concordance decreased with increasing age, we could show here that this was more an effect of decreasing GFR with ageing than age itself [34]. Regarding the potential discordances between the single- and multiple-sample methods according to GFR level, it is obvious that the most important discordances were observed at low GFR levels (<30 mL/min). When GFR was >30 mL/min, the 240 min time point, or, even slightly better, a strategy of choice depending on the expected GFR, gave very acceptable concordances. The differences of concordance for GFR <30 mL/min should be interpreted with caution. First, only 32 patients with four samples available had GFR <30 mL/min. Secondly, the differences between the two methods were systematic (the single sample giving higher results), suggesting that a systematic, and thus methodologic bias explains the difference. The lack of a late (or very late) time point is a probable reason to explain the difference between the two methods [11, 13, 35–37]. In our analysis, the 120 min time point was definitely the least accurate. Also, the 180 min time point was usually less accurate than the 240 min time point, except in the patients with high GFR levels (GFR >130 mL/min). However, in such patients with high GFR level, the concordance within 10% at 240 min was also high (95%) and may be the optimal time point also at higher GFR, since in clinical practice, it can be difficult to choose *a priori* (i.e. before measuring GFR) the correct time point, as most of estimating GFR equations systematically underestimate very high GFR values [38].

Regarding BMI categories, the discordances were mainly relevant in the highest BMI category (BMI ≥40 kg/m^2). Concordance between the two methods remained acceptable in all other categories if the 240 min time point (or the strategy according to GFR levels) was used. Weight is part of the single-sample method, being an important variable of the equation used to estimate extracellular volume by Granerus equation (see Supplementary Data). The Granerus equation is also an estimation and may be less accurate and a source of bias at very high BMI levels. Contrary to differences due to GFR levels, the differences between single- and multiple-sample clearances according to BMI category are not systematic.

To the best of our knowledge, this is the largest study ever having compared the concordance between single- and multiple-sample plasma clearance methods [13, 16–19, 35, 36, 39–46]. Focusing on prior adult studies with a large sample and adequate statistics (bias, precision and concordance/accuracy), we confirm and extend prior findings [15–19]. In 1996, Gaspari *et al.* compared iohexol single sample (at 240 min for GFR ≥40 mL/min and 450 or 600 min for GFR <40 mL/min) with a multiple-sample method (time point at 120, 180 and 240 min for GFR ≥40 mL/min, and 300, 450 and 600 min if GFR <40 mL/min) in 686 patients. Bias and precision were similar to those we observed. However, the authors found an accuracy within 5% slightly lower than ours (75%) and also a significant regression intercept between the two methods [15]. Like Gaspari *et al.* we also found that concordance was the best when the time point was 240 min for GFR ≥40 mL and 180 min for highest GFR levels. However, the concordance within 5% was better in our study and, more importantly, we did not find any significant regression intercept between the two methods with a very high concordance correlation coefficient. Sterner *et al.* in 65 CKD patients [16], Lundqvist *et al.* in 902 subjects admitted for urography [19], Brändström *et al.* in 46 CKD patients [18], and Bird *et al.* in 56 CKD patients and 19 healthy subjects [17] showed comparable acceptable biases between single- and multiple-sample methods. Brändström and Bird showed equivalent results for ^{51}Cr-EDTA single-sample method [17, 18]. For Lundqvist and Bird, the best results were observed, as in our analysis, when the time point of the single sample was at 240 min, compared with earlier time points [17, 19]. In summary, we can extend conclusions of prior studies showing that 240 min is the best time point for single sample, especially when GFR is >50 mL/min. Our proposed strategy to consider a later timing (at 300 min or later) if expected GFR or eGFR is <50 mL/min/1.73 m^2 seems to further improve the concordance between the single- and multiple-sample methods. However, only few patients underwent very late sampling (450, 600 or even 1440 min), and, therefore, we cannot test the hypothesis that these very late time points would be useful at very low GFR levels (<30 mL/min) [11, 13, 35, 36, 47]. There are other limitations to our study. In the current work, ethnicity was not available in our cohort, even if there is no obvious reason for assuming that ethnicity would explain discrepancies between single- and multiple-sample methods. Furthermore, we only considered the single-sample method as proposed by Jacobsson [21]. Other mathematical models have been proposed [36, 48–53], but Jacobsson's method is clearly the most widely used. Finally, we discussed concordance, not accuracy, between single- and multiple-sample methods. Indeed, considering the multiple-sample method the reference method remains questionable, as no gold standard reference method

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(such as inulin urinary clearance) was available [13, 16, 54]. This limitation could be especially relevant in low GFR ranges or in patients with increased V_d (ascites or oedema). In these clinical scenarios, it is plausible that both single- and multiple-sample methods are not accurate, especially in the absence of a late (or very late) sample [8, 9, 11, 55].

Iohexol plasma clearance is the method to measure GFR with the best balance between physiology and feasibility. However, there is still a lack of standardization, like for other GFR measurement methods. In the current analysis, we showed that single- and multiple-sample clearances are concordant, and this observation was also applicable to ^{51}Cr-EDTA. Some relevant discrepancies were also observed in subjects with very high BMI ($\geq$40 kg/m^2) and in patients with low GFR (<30 mL/min). The main limitation of the single sample is, by definition, the unique sample. In all plasma procedures, potential random analytical errors (especially with the last time points where concentrations of the tracer can be low), blood drawing or timing errors may sometimes occur. From multiple-sample results, outlier points can easily be identified by observing the declining concentration–time curve. This has been the case in the current analysis where 4.5% of the multiple-sample results were excluded because goodness of fit was judged insufficient (by the stringent criterion of $R^2 < 0.975$). In clinical practice, outlier points can thus be discarded (or measurement be repeated) when using the multiple-sample method. There is no such internal quality control with the single-sample method [8].

CONCLUSION

In conclusion, performances of a single-sample method are reasonably concordant with a multiple-sample method and can be considered as an alternative tool of plasma clearance evaluation of GFR. As a result of our work, we would recommend a single sample at 240 min if the expected GFR or eGFR is >50 mL/min/1.73 m^2 and at 300 min or later if the expected GFR or eGFR is <50 mL/min/1.73 m^2. Such an approach may be of particular interest for large epidemiological studies or clinical trials, where a convenient technique with limited cost is required [2, 8, 56].

AUTHORS' CONTRIBUTIONS

P.D. provided research idea, designed the study and was responsible for drafting the article. P.D., M.F., L.D., E.V.-P., S.L., E.S., N.E. and H.P. carried out the data analysis and interpretation. P.D., M.F., L.D., E.V.-P., E.S. N.E. and H.P. carried out the data acquisition. H.P. provided the statistical analysis. M.F., L.D., E.V.-P., L.S., E.C., E.S., N.E. and H.P. assisted in revising the article. M.F., L.D., E.S. and N.E. carried out the supervision of the manuscript.

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SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

CONFLICT OF INTEREST STATEMENT

N.E. and E.S. report grants from KfH Foundation for Preventive Medicine, during the conduct of the study. The other authors declare that they have no relevant financial interests.

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