Effect of extracellular fluid volume on single-sample measurement of glomerular filtration rate

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Abstract
Objective. The objective of our study was to evaluate the effect of extracellular fluid volume (ECV) on the accuracy of measurement of glomerular filtration rate from a single sample (GFR1).

Methods. Multi-sample GFR (GFR6) and ECV (per 1.73 m²) were measured with both Cr-51-EDTA and iohexol, injected into opposite arms (110 studies in 80 subjects). Six plasma samples were obtained bilaterally 20–240 min post-injection to measure GFR6/1.73 m². GFR1/1.73 m² was calculated from 2-, 3- and 4-h samples using Jacobsson’s formula for iohexol and the Christensen and Groth formula for Cr-51-EDTA. The quotient, GFR1/GFR6, was taken to indicate the accuracy of GFR1.

Results. When GFR6 was <60 ml/min/1.73 m², GFR1/GFR6 correlated positively with ECV at all single-sample times. When GFR6 was 60–90 ml/min/1.73 m² or >90 ml/min/1.73 m², GFR1/GFR6 correlated positively with ECV at 2 h, but negatively at 4 h, indicating that at some time between 2 and 4 h, GFR1/GFR6 was transiently independent of ECV. A plot of the regression gradient of GFR1/GFR6 on ECV against sample time indicated that the time of transient independence, at which GFR1 depends exclusively on GFR6, was 3.2–3.9 h (depending on indicator combination used) when GFR6 was 60–90 ml/min/1.73 m² and 2.4–2.9 h when GFR was >90 ml/min/1.73 m². Transient independence when GFR6 was <60 ml/min/1.73 m² was not reached by 4 h and estimated to be 5–7 h.

Conclusion. The accuracy of GFR1 depends on ECV, over-estimation or underestimation respectively depending on sample time and GFR. The time at which GFR1 is independent of ECV increases with decreasing GFR. If sampling time is too early, GFR1 overestimates GFR, but the reverse occurs when sampling is too late, even if GFR is abnormally low.

Keywords: Cr-51-EDTA; glomerular filtration rate; iohexol; single sample

Introduction
In Europe, clinical measurement of glomerular filtration rate is usually with Cr-51-ethylenediaminetetraacetic acid (EDTA) or iohexol [1]. Rather than obtaining multiple samples starting soon after injection, plasma clearance measurement is routinely based on blood samples obtained from 2 h, an approach known as the slope-intercept technique. A further simplification is to measure GFR from the concentration of indicator recorded in a single sample at a specified time after injection (GFR1) [2–17]. Several formulae have been described that relate this concentration to true GFR. They generally take into account the patient’s extracellular fluid (ECF) volume (ECV), assumed to be a fixed proportion of body weight or surface area.

Theoretically, the accuracy of GFR1 depends on (a) the rate of indicator mixing throughout the ECV, (b) ECV itself and (c) GFR. It is widely assumed that mixing rate is invariable between subjects and this is the basis of one-compartment correction formulae, such as that of Brochner-Mortensen [18], used in the slope-intercept technique. Less, however, is known about the effects of variations in ECV. In this study, therefore, we examined the effect of ECV on the discrepancy between GFR1 and GFR conventionally measured from multiple samples.

Methods

Subjects
Dual simultaneous and independent measurements of GFR and ECV with Cr-51-EDTA and iohexol were undertaken in 80 subjects, comprising 60 patients referred for routine investigation (40 males, 20 females, age range 28–79 years, BMI 20–41 kg/m²) and 20 healthy subjects (7 males, 13 females, age range 30–59 years, BMI 18–34 kg/m²) with no history of allergy to iodine-containing contrast agents. Of the patients, 36 were diabetics, 10 had cancer, 13 had skin disease and were receiving or being considered for cyclosporine treatment and one was referred from the nephrology service. Of the 20 healthy volunteers, 10 were studied twice and 10 studied three times, as part of a reproducibility
study published elsewhere [19], giving a total of 110 dual measurements. All subjects gave written informed consent and the study was approved by the local research ethics committee and Administration of Radioactive Substances Advisory Committee of the United Kingdom.

**Procedure**

Cr-51-EDTA and iohexol (Omnipaque 300) were obtained from GE healthcare, Bucks, UK. Syringes were weighed before and after drawing up the markers. Two millilitres of the Cr-51-EDTA (2 MBq) and iohexol (19 ml containing 5.7 g iodine) were separately injected into each ante-cubital fossa and 10 ml samples drawn bilaterally before and 20, 40, 60, 120, 180 and 240 min after injection. The syringes were flushed several times with saline to ensure administration of all markers.

**Sample analysis**

Plasma, standard and water (as a blank) samples were assayed for Cr-51 in an automatic gamma counter (Wallac 1480 Wizard 3rd, Turku, Finland) for 1000 s. Appropriate corrections were made for counter dead time and background. Iohexol was assayed by x-ray fluorescence (XRF; Oxford Instruments, Oxford, UK). Each marker was assayed in samples drawn contralateral to the side it was injected.

**Data analysis**

**Measurement of GFR.** Plasma clearance curves of both indicators were resolved into two exponentials with rate constants $\alpha_1$ and $\alpha_2$ and corresponding zero-time intercepts $A$ and $B$ using a two-stage curve-stripping procedure, as previously described [19]. Multi-sample GFR (GFR6) was calculated using the following conventional formula for both Cr-51-EDTA (GFR6EDTA) and iohexol (GFR6iohexol):

$$\text{GFR} = \frac{\text{injected activity or dose}}{(A/\alpha_1) + (B/\alpha_2)}$$

In five studies, the iohexol clearance curve could not be resolved into two exponentials so they were excluded.

Body surface area (BSA) was calculated from height and weight [20] and GFR scaled to 1.73 m$^2$.

**Measurement of ECV.** Indicator distribution volume, assumed to equal ECV, was calculated from multi-sample clearance using the equation described by Nosslin [21]:

$$T = \frac{(A/\alpha_1)^2 + (B/\alpha_2)^2}{(A/\alpha_1) + (B/\alpha_2)}$$

where $T$ is the mean residence time of indicator molecules in the ECF space before filtration. $1/T$ is equal to GFR per unit ECV, so $1/T$ multiplied by GFR6 is ECV. Like GFR1 and GFR6, ECV was scaled to a BSA of 1.73 m$^2$.

**Measurement of single-sample GFR.** Single-sample GFR (GFR1) was calculated from marker concentration in specifically timed plasma samples close to 2, 3 and 4 h and scaled to a BSA of 1.73 m$^2$. Apart from studies designed to develop new formulae, single-sample methods based on Cr-51-EDTA have often used the Christensen and Groth formula [7] while those based on iohexol have often used the Jacobsson formula [4], so for Cr-51-EDTA we also used the Christensen and Groth formula and for iohexol we used the Jacobsson formula. Neither of these formulae restricts sampling to a specific time post-injection. With respect to the Jacobsson formula, ECV was estimated for men and women separately, as described by Gaspari et al. [15].

**Statistics**

Subject studies were divided into three groups based on GFR6: <60, 60–90 and >90 ml/min/1.73 m$^2$. Agreement between GFR1 and GFR6 was expressed as their quotient, which was then regressed by linear least-squares analysis on ECV. This was done for each of the three time points and each of the three function categories, giving nine regression slopes (see Figure 1). With the simultaneous use of two markers, there were two measures each of GFR1, GFR6 and ECV, giving four sets of nine regression slopes of GFR1/GFR6 on ECV. Within each set, the regression gradient was plotted as a function of sample time for each of the three function categories. The times at which the three plots (one for each function category) crossed the zero line were recorded (see Figure 2). These times are the points at which GFR1, as a predictor of true GFR, is transiently independent of ECV and therefore, mixing time aside, exclusively dependent on GFR6.

**Results**

GFR6EDTA $(n = 110)$ was <60 ml/min/1.73 m$^2$ in 24 subject studies, 60–90 ml/min/1.73 m$^2$ in 40 and >90 ml/min/1.73 m$^2$ in 46. GFR6iohexol $(n = 105)$ was <60 ml/min/1.73 m$^2$ in 20 subject studies, 60–90 ml/min/1.73 m$^2$ in 53 and >90 ml/min/1.73 m$^2$ in 32.

The relations between GFR1/GFR6 and ECV (when all were measured with Cr-51-EDTA) are shown in Figure 1(A–C). When GFR6 was <60 ml/min/1.73 m$^2$, GFR1/GFR6 correlated positively and significantly with ECV at all three time points. When GFR6 was >90 ml/min/1.73 m$^2$, the gradient of the regression slope was positive at 2 h but negative at 4 h. Thus, when GFR6 was <60 ml/min/1.73 m$^2$, GFR1 increasingly overestimated GFR6 as ECV increased, irrespective of the time on which GFR1 was based. In contrast, when GFR6 was >90 ml/min/1.73 m$^2$, GFR1 increasingly underestimated GFR6 with increasing ECV when GFR1 was based on 2 h, but showed the opposite relation when GFR1 was based on 4 h.

The regression slopes of the individual relations between GFR1/GFR6 and ECV (all measured with Cr-51-EDTA) are shown in Figure 2A as a function of sample time for each of the three function categories. Thus, for all three function categories, the regression gradient decreased as sample time increased. Similar patterns of slope versus sample time were seen for all four combinations of markers with which GFR1/GFR6 and ECV were measured (Figure 2A). Similar slope versus time patterns were
observed when the population was split between patients and normal volunteers (Figure 2B).

A regression slope of zero with respect to the relations between GFR1/GFR6 and ECV, illustrated in Figure 1, indicates that GFR1 is independent of ECV and, by implication, is exclusively dependent on GFR6. For all four panels in Figure 2A, regression slopes were always much greater than zero when GFR6 was <60 ml/min/1.73 m² and, judging by forward extrapolation, would not have reached zero until a sampling time of 5–7 h. For studies in which GFR6 was 60–90 ml/min/1.73 m², the sampling times at which the regression slopes would have been zero ranged from 3.2 to 3.6 h. For studies in which GFR6 was >90 ml/min/1.73 m², the corresponding range was 2.4–2.9 h.

Discussion

Most previous papers on single sample measurement of GFR have commented on the importance of sample time in relation to prevailing filtration function [2,6,13]. Nevertheless, although in some cases influence of sample timing has been incorporated in a theoretical framework [2], there is a paucity of experimental evidence that explains this relationship. Our study, therefore, is useful in that it illustrates, in a novel way, how the closeness with which single sample GFR predicts multi-sample GFR depends on ECV. Theoretically, the timed plasma marker concentration, and therefore its accuracy in the determination of true GFR, depends on three factors—the rate of mixing of marker throughout the ECF, ECV itself and GFR. The rate of mixing bears complex relations with ECV and GFR, but determines how much marker is available for filtration over the period of mixing, normally ~2 h. Delay in mixing would result in overestimation of true GFR by GFR1.

ECV has several effects. Firstly, an expanded ECV promotes delayed mixing. Secondly, ECV determines the marker concentration reached according to simple marker dilution and when expanded will through this mechanism lead to overestimation of true GFR. Thirdly, ECV

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Fig. 1. Relation between the quotient GFR1/GFR6 and ECV, all measured with Cr-51-EDTA, when GFR1 was based on 2, 3 or 4 h for subject studies in which GFR6EDTA was (A) <60 ml/min/1.73 m² (open circles); (B) 60–90 ml/min/1.73 m² (closed circles) and (C) >90 ml/min/1.73 m² (open squares). Regression lines [for significant \( P < 0.05 \) relations] and equations are shown.
Fig. 2. Relation between regression slope of GFR1/GFR6 on ECV (as illustrated in Figure 1) and time of single sample for (A) all subject studies in which GFR6 was <60 ml/min/1.73 m² (open circles), 60–90 ml/min/1.73 m² (closed circles) and >90 ml/min/1.73 m² (open squares) for various indicator combinations (ECV measured with Cr-51-EDTA in left panels and with iohexol in right panels; GFR measured with Cr-51-EDTA in upper panels and with iohexol in lower panels) and (B) patient and normal volunteer studies shown separately (only for GFR and ECV both based on Cr-51-EDTA; no normal subject study had a GFR6 <60 ml/min/1.73 m²). The time at which GFR1 becomes transiently independent of ECV is the time at which the relation cuts the horizontal dashed line. This time increases as GFR6 increases.

Fig. 3. Hypothetical examples of plasma indicator clearance curves of two subjects who although they have the same GFR have different ECF volumes, illustrating different indicator concentration–time curves. It can be seen that GFR1 simultaneously depends on GFR and ECV. Thus, GFR1, which is determined by the virtual distribution volume of the indicator at the sampling time, would be lower in the subject illustrated by the bold line (with the lower ECV) at 120 min but higher at 240 min. Note that the subject illustrated by the bold line (lower ECV) has earlier completion of mixing and higher terminal rate constant.

determines the turnover rate of marker (or its average residence time in the ECV). An expanded ECV will result in a reduction in the terminal plasma clearance rate constant (close to GFR per unit ECV) and, with the passage of time, lead to progressive underestimation of true GFR by GFR1.

These opposing effects of ECV are illustrated in Figure 3, which shows a hypothetical comparison between two subjects with identical values for GFR but different ECF volumes. In the subject with the higher ECV, the 2 h single-sample value would overestimate GFR because of delayed mixing and marker dilution. The rate of clearance of the marker by filtration would however then be slower because of a reduction in GFR per unit ECV, so marker plasma concentration would fall more slowly and the single-sample method progressively underestimate GFR the later the sample was taken. At a specific sampling time point, however, single-sample GFR would become transiently independent of ECV. This time point was 3.2–3.6 h for subjects with GFR6 in the range 60–90 ml/min/1.73 m² and 2.4–2.9 h for those with GFR6 >90 ml/min/1.73 m². For subjects with GFR6 <60 ml/min/1.73 m², the ECV-neutral time point was not reached but, by extrapolation, could be estimated to be 5–7 h. For patients with poor renal function (e.g. <30 ml/min/1.73 m²), it can be predicted that single-sample GFR is highly dependent on ECV over the first few hours after marker injection. This has been illustrated in several studies including that of Sterner et al. [13] who showed empirically that the best sampling time was 7 h for patients with GFR of 20–50 ml/min and 24 h when GFR was <20 ml/min. Nevertheless, even for such renally impaired patients, it is perfectly feasible for sampling time to be too late.

Guidelines for the use of single-sample methods are difficult to define owing to the unpredictable character of factors influencing the determination of GFR. The current study demonstrates the impact of one such factor, namely ECF volume, potential variability of which may adversely affect the accuracy of the single-sample technique and which explains why sampling time has to be delayed in patients with poor function. For users of the single-sample technique, it can nevertheless be concluded that when GFR is anticipated to be >60 ml/min/1.73 m², the sample should be obtained at about 3 h because, at this time, GFR1 has no dependence on ECV. When GFR is anticipated to be <60 ml/min/
1.73 m², however, sample timing is much more dependent on ECV at early time points, including 4 h, and should be delayed beyond 4 h, depending on function. It is important to note, however, that delaying it too long for the prevailing level of filtration function will re-introduce the dependence of GFR1 on ECV. It is therefore recommended that in these subjects, a conventional multi-sample approach should be considered.

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References


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