



## CKJ REVIEW

# Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review.

## Part 1: How to measure glomerular filtration rate with iohexol?

Pierre Delanaye<sup>1</sup>, Natalie Ebert<sup>2</sup>, Toralf Melsom<sup>3,4</sup>, Flavio Gaspari<sup>5</sup>, Christophe Mariat<sup>6</sup>, Etienne Cavalier<sup>7</sup>, Jonas Björk<sup>8</sup>, Anders Christensson<sup>9</sup>, Ulf Nyman<sup>10</sup>, Esteban Porrini<sup>11</sup>, Giuseppe Remuzzi<sup>12,13</sup>, Piero Ruggenenti<sup>12,13</sup>, Elke Schaeffner<sup>2</sup>, Inga Soveri<sup>14</sup>, Gunnar Sterner<sup>15</sup>, Bjørn Odvar Eriksen<sup>3,4</sup> and Sten-Erik Bäck<sup>16</sup>

<sup>1</sup>Department of Nephrology, Dialysis and Transplantation, University of Liège Hospital (ULg CHU), Liège, Belgium, <sup>2</sup>Charité University Medicine, Institute of Public Health, Berlin, Germany, <sup>3</sup>Metabolic and Renal Research Group, UiT The Arctic University of Norway, Tromsø, Norway, <sup>4</sup>Section of Nephrology, University Hospital of North Norway, Tromsø, Norway, <sup>5</sup>IRCCS - Istituto di Ricerche Farmacologiche 'Mario Negri', Centro di Ricerche Cliniche per le Malattie Rare 'Aldo e Cele Daccò', Ranica, Bergamo, Italy, <sup>6</sup>Department of Nephrology, Dialysis, Transplantation and Hypertension, CHU Hôpital Nord, University Jean Monnet, PRES Université de LYON, Saint-Etienne, France, <sup>7</sup>Department of Clinical Chemistry, University of Liège Hospital (ULg CHU), Liège, Belgium, <sup>8</sup>Department of Occupational and Environmental Medicine, Lund University, Lund, Sweden, <sup>9</sup>Department of Nephrology, Skåne University Hospital, Lund, Sweden, <sup>10</sup>Department of Translational Medicine, Division of Medical Radiology, Skåne University Hospital, Malmö, Sweden, <sup>11</sup>University of La Laguna, CIBICAN-ITB, Faculty of Medicine, Hospital Universtario de Canarias, La Laguna, Tenerife, Spain, <sup>12</sup>Centro di Ricerche Cliniche per le Malattie Rare 'Aldo e Cele Daccò, Istituto di Ricerche Farmacologiche Mario Negri, Centro Anna Maria Astori, Science and Technology Park Kilometro Rosso, Bergamo, Italy, <sup>13</sup>Unit of Nephrology, Azienda Socio Sanitaria Territoriale (ASST) Ospedale Papa Giovanni XXIII, Bergamo, Italy, <sup>14</sup>Department of Medical Sciences, Uppsala University, Uppsala, Sweden, <sup>15</sup>Department of Nephrology, Skåne University Hospital, Malmö, Sweden and <sup>16</sup>Department of Clinical Chemistry, Skåne University Hospital, Lund, Sweden

Correspondence and offprint requests to: Pierre Delanaye; E-mail: pierre\_delanaye@yahoo.fr

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## Abstract

While there is general agreement on the necessity to measure glomerular filtration rate (GFR) in many clinical situations, there is less agreement on the best method to achieve this purpose. As the gold standard method for GFR determination, urinary (or renal) clearance of inulin, fades into the background due to inconvenience and high cost, a diversity of filtration markers and protocols compete to replace it. In this review, we suggest that iohexol, a non-ionic contrast agent, is most suited to replace inulin as the marker of choice for GFR determination. Iohexol comes very close to fulfilling all requirements for an ideal GFR marker in terms of low extra-renal excretion, low protein binding and in being neither secreted nor reabsorbed by the kidney. In addition, iohexol is virtually non-toxic and carries a low cost. As iohexol is stable in plasma, administration and sample analysis can be separated in both space and time, allowing access to GFR determination across different settings. An external proficiency programme operated by Equalis AB, Sweden, exists for iohexol, facilitating interlaboratory comparison of results. Plasma clearance measurement is the protocol of choice as it combines a reliable GFR determination with convenience for the patient. Single-sample protocols dominate, but multiple-sample protocols may be more accurate in specific situations. In low GFRs one or more late samples should be included to improve accuracy. In patients with large oedema or ascites, urinary clearance protocols should be employed. In conclusion, plasma clearance of iohexol may well be the best candidate for a common GFR determination method.

**Key words:** glomerular filtration rate, iohexol

## Introduction

Since Homer W. Smith published in 1951 his famous textbook 'The Kidney: Structure and Function in Health and Disease', glomerular filtration rate (GFR) has been considered as one of the best physiologic measures of kidney function [1]. The historical gold standard for GFR measurement, which is urinary clearance of inulin, is however difficult to perform in practice. Indeed, these pioneers of nephrology performed repeated urine collection every 10 or 15 min with a urinary catheter, a constant infusion of inulin and arterial samples [1, 2]. Several alternatives such as isotopic ( $^{125}\text{I}$ -iothalamate,  $^{51}\text{Cr}$ -EDTA or  $^{99\text{Tc}}$ -DTPA) or non-isotopic (iohexol or iothalamate) 'cold' markers have thus been proposed to measure GFR [3]. Importantly, the term 'alternative' also implies that another methodology than urinary clearance can be used, i.e. plasma clearance [4–6].

Estimated GFR by equations based on creatinine and/or cystatin are less accurate than measured GFR. Although equations for estimating GFR are useful, especially outside nephrology, the precision of the equations can be relatively poor in specific clinical situations (see Part 2 of the article) [7–9]. Also, both creatinine and cystatin C are influenced by non-GFR factors [10–12]. This has been demonstrated in several and diverse clinical populations, i.e. diabetes mellitus, chronic kidney disease (CKD), renal and non-renal transplantation, patients with cirrhosis, heart failure and also in healthy subjects [13–18]. Thus, in many clinical contexts a measured GFR is required.

In Part 1 of this review, we will focus on iohexol plasma clearance, the most popular method used to measure GFR in Europe. We will review general properties, safety aspects, analytical considerations, agreement with other procedures and current available procedures of iohexol plasma clearance. In Part 2, we focus on the role of iohexol plasma clearance both in clinical practice and research.

## Iohexol: general properties and safety

Iohexol is a non-ionic contrast medium, a principle developed by the Swedish radiologist Torsten Almén [19, 20]. It is mainly used for computed tomography (CT), catheter-based angiography and interventions. The first description of iohexol in humans was published in 1980 [21]. Increasing doses of iohexol (125–500 mg I/kg) were injected into 20 healthy subjects. In this seminal study, it was shown that the substance was safe and

fully excreted by the kidneys. In 10 subjects, it was additionally shown that iohexol did not affect GFR measured by  $^{51}\text{Cr}$ -EDTA. The authors mentioned that urinary clearance of iohexol was higher than urinary clearance of  $^{51}\text{Cr}$ -EDTA, but no additional details were given [21]. In 1983, data from this first study were re-analysed [22]. Iohexol total clearance was identical to  $^{51}\text{Cr}$ -EDTA, whereas urinary clearance of iohexol was slightly higher. Finally, they showed that iohexol was distributed within the extracellular volume [22]. This iohexol distribution pattern applies also to patients with CKD and obese subjects [22–26]. Importantly for a GFR marker, other studies demonstrated that iohexol had no effect on the GFR level *per se* [21, 27].

The molecular weight of iohexol is 821 Da [22, 28]. The proportion of iohexol bound to protein appears to be very low. The first study described a binding to protein of only 1.5% [29], which was also confirmed by others [26, 30, 31], although one recent publication challenged these findings [32]. Regarding its molecular weight and the absence of binding to proteins, there is little doubt that this marker is freely filtered through the glomerulus.

The question of the extra-renal clearance of a marker is important. Briefly, this question can be studied by two different methodologies: either measuring the plasma clearance in anephric patients (or in patients with very low residual function) or measuring plasma and urinary clearances, the difference between both being the extra-renal clearance. Extra-renal clearance of iohexol is low with both methodologies. Studying anephric patients, extra-renal clearance of iohexol was between 2 and 3 mL/min/1.73 m<sup>2</sup> [24, 33–35]. Studying the difference between plasma and urinary clearances in healthy subjects, the extra-renal clearance of iohexol was between 0 and 6 mL/min/1.73 m<sup>2</sup> [22, 26, 30, 36] or 5% [25]. These results must be compared with extra-renal clearance of other markers such as iothalamate and  $^{51}\text{Cr}$ -EDTA, which has been found to be between 4 and 10 mL/min/1.73 m<sup>2</sup> for iothalamate [26, 37–39] and around 2–4 mL/min/1.73 m<sup>2</sup> for  $^{51}\text{Cr}$ -EDTA [40, 41].

To be considered a reference marker for measuring GFR, there should not be any tubular secretion or absorption. Iohexol differs from iothalamate in being non-ionic, and active secretion or absorption of iohexol has to our knowledge not been demonstrated. In contrast, a concentration gradient dependent tubular transport of iothalamate and other ionic contrast media is well documented in several species, including human [42, 43].

The safety of iohexol has been extensively studied [44, 45] and is confirmed by the large number of iohexol measurements

performed in countries, e.g. Sweden (in Skåne county) (~1500 GFR measurements/year) [46, 47] and Italy (25 000 iohexol clearances since 1995 at the Clinical Research Center of Mario Negri Institute in Bergamo) (F.G., personal data). No severe adverse event, and particularly no anaphylactic reaction, occurred. This safety profile is, at least in part, explained by the current low dose of iohexol injected (5 or 10 mL versus 80–180 mL for CT scan and 130–300 mL for coronary interventions) and by the exclusion of patients with known contrast medium reactions. Even in patients with minimal renal function, iohexol doses of 10 mL (300 mg I/mL) have not been shown to be nephrotoxic by measuring serum creatinine but also urinary N-acetyl-beta-D-glucosaminidase and  $\alpha$ 1-microglobulin, at 1 and 4 days after injection [48]. Altogether, the co-authors of this review article have performed around 10 000 iohexol GFR measurements over the last 2 years. Anaphylactic shock never occurred.

## Iohexol: analytical and other metrics considerations

### Analytical considerations

Iohexol can be measured using different methods. High performance liquid chromatography with ultraviolet detection (HPLC-UV), X-ray fluorescence (XRF) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are certainly the most validated methods. There are other assays, e.g. capillary electrophoresis, but such methods have been poorly validated, and nowadays are seldom used [49, 50]. HPLC-UV is clearly the most commonly used method in Europe and was historically the first method described [21] to quantify iohexol [30]. Measurement of iohexol by HPLC-UV is sensitive, specific and reproducible, enabling the use of minute doses of iohexol, as low plasma concentrations can be accurately quantified [26, 30, 45, 51–59]. The high sensitivity also allowed the development of finger-prick samples (capillary sampling). However, this capillary technique needs to be developed further, as the precision of this method cannot yet be considered sufficient [60–65]. Measurement of iohexol by XRF has been well described [45, 66–72] but requires specific instrumentation. Few studies have compared these two methods for GFR measurement purposes [69], yet these data indicate that the performance of XRF is inferior to HPLC-UV due to its lower sensitivity [69, 73]. Measurement of iohexol by LC-MS/MS has been more recently described by several authors [32, 74–79]. LC-MS/MS is theoretically a more sensitive and specific method but is more complex and costly compared with HPLC-UV.

A fundamental question is whether differences in iohexol measurement methodology will affect GFR results, as has been shown for other markers like inulin or iothalamate [80, 81]. Recent data suggest a high concordance between iohexol concentrations and plasma clearances measured by HPLC-UV or LC-MS/MS in 102 CKD and healthy subjects, but a systematic correction factor of 10% must be applied [82]. Other authors performed an inter-laboratory comparison of iohexol measurement. In 20 paediatric patients, they compared iohexol concentrations (range 15–700  $\mu$ g/mL) measured by LC-MS/MS in the USA with those determined on the same samples by HPLC-UV by a European reference laboratory in Italy. The concordance between the two methods was very high both for iohexol concentrations and GFR results (difference of <10% between the two laboratories, except for one patient) [83]. Further research is still required regarding the effect of the measurement method (HPLC or LC-MS/MS) on iohexol (or iothalamate) clearance results.

Two very important analytical points should be highlighted when discussing GFR measurement. Firstly, iohexol is perfectly stable at room temperature,  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  [32, 84]. Iohexol clearance investigations can therefore be performed in virtually all medical settings. Samples can be measured at a tertiary care hospital and then shipped to a central laboratory. No shipment in dry ice is needed. The high stability of iohexol is a major advantage in comparison with isotopic methods that can be performed only in nuclear medicine units and for which samples are difficult to store.

Secondly, an external quality assurance programme does exist, provided by Equalis AB, Uppsala, Sweden. Two serum or plasma samples with added iohexol are distributed to participants four times per year. Additional data (patient gender, height and weight for body surface calculation, administered dose of iohexol and timing of samples) are provided to enable programme participants to calculate iohexol clearance using a one- or two-sample calculation (or both). This proficiency programme includes both HPLC-UV (the majority) and LC-MS/MS measurements. Each laboratory can compare its own results to that of other participating laboratories. In 2015, 35 laboratories participated in this programme. Agreement between overall means and spiked concentrations is high. Between-laboratory reproducibility for each survey is ~5% [coefficient of variation (CV)]. Analysis of variance calculated from repeated distribution of the same pools over a 3-year period yields a within-laboratory reproducibility of 5%, a between-laboratory reproducibility of 3–4% and a total variation of 5–7%. The different methods of iohexol analysis perform equally well. To the best of our knowledge, such an external quality programme does not exist for other GFR markers wherefore less knowledge is available about their inter-laboratory variation [56].

### Intra-individual variation

The biological variation of any physiological quantity should be considered in the interpretation of biological results. The biological CV, represents the 'normal' variation of a parameter that can be observed in the same individual. GFR is influenced by several physiological parameters such as diet, activity or circadian variations [71, 85–87]. In the case of measured GFR, intra-individual variation includes both biological variation and the errors due to the measurement of GFR itself. Variation is thus dependent on both the markers and measurements protocols [88]. Ideally, the GFR measurement should be performed using a standardized procedure, e.g. measurement in a fasting state, in the same environment and at the same time of day, because all these parameters are prone to increase intra-individual variation. Intra-individual variation can also differ according to the population tested. For example, intra-individual variation may be higher in CKD patients than in a healthy population [89], even if this is not found by all authors [6]. Literature gives an intra-individual variation of measured GFR (independently of the marker considered) of 4.2 to 10% [6, 23, 30, 36, 71–73, 86, 88–99]. This means that in the same given individual, GFR must increase or decrease by at least 5–15% (depending on the intra-individual CV of the method used) to be considered clinically relevant. CV of 10% is frequently considered and should be kept in mind when analysing studies comparing different GFR measurement methods, or estimated versus measured GFR. In such studies, accuracy within 10 or 30% is frequently used. Accuracy within 10% is the percentage of results with one method that are within  $\pm 10\%$  of the results of another method, and can, with respect to intra-individual variation of GFR, be considered as an excellent agreement between the methods.

**Table 1.** Examples of GFR variability with different iohexol procedures

Author Reference	Sample	Protocol	Population	GFR variability (CV)
Krutzen [30]	9	PC: samples at 120 and 240 min + BM correction	Healthy	11.4%
Delanaye [73]	12	PC: samples at 120 and 240 min + BM correction	Healthy	4.5%
Eriksen [99]	88	PC: single-sample + Jacobsson correction	General population	4.2%
Gaspari [6]	24	PC: samples at 120, 150, 180, 210 and 240 if eGFR >40 mL/min and at 120, 180, 240, 300, 450 and 600 min if eGFR <40 mL/min + BM correction	Healthy and CKD	5.6%

eGFR, estimated glomerular filtration rate; CV, coefficient of variation; CKD, chronic kidney disease; PC, plasma clearance; BM, Brochner-Mortensen [116].

In Table 1, we summarize estimates of intra-individual variability published by co-authors of the current article when GFR is measured with iohexol.

### Iohexol: studies comparing iohexol with inulin clearance

To the best of our knowledge, only two studies have compared urinary clearances of iohexol and inulin [100, 101] (Table 2). Both showed excellent correlation between the two methods, but the statistical tools used in the original publications were limited and re-analysed data demonstrated less impressive concordance [3]. On the other hand, several studies have compared iohexol plasma clearances with urinary inulin clearance [52, 102–106]. In general, these studies showed a good correlation between the methods. Results are less accurate in the study published by Erley *et al.*, but the patients included were hospitalized in an intensive care unit [104]. The value of plasma clearance (whichever marker) in this setting is probably questionable because the extracellular volume, and consequently the volume of distribution of the marker, is quite variable from patient to patient and within the same patient at different points due to changes in fluid infusions and in biological fluid loss or sequestration in different compartments [104, 107]. Another study did not describe the slope-intercept method used [102]. The studies published by Gaspari *et al.* in adults [52] and Berg *et al.* in children [105] are more relevant from a methodological point of view (see Table 2 for details). They showed low bias and relatively good precision between the two methods. Both studies included a large number of samples including a late sample (600 and 1440 min), the last point being of importance as it will be described further in the text [52, 105].

In a recent review article [3], authors pooled data from seven available studies comparing iohexol (both plasma or urinary clearance) with GFR measured by inulin and calculated median bias and accuracy within 30 and 10%. For iohexol urinary clearance studies, the mean ( $\pm$  standard deviation) GFR, measured by inulin, was  $81 \pm 38$  mL/min/1.73 m<sup>2</sup>. Median bias (corresponding to the median difference between iohexol and inulin) was  $-7\%$  [95% confidence interval (CI):  $-10$  to  $0$ ]. Pooled accuracy within 30% was 100% (CI not reported) and accuracy within 10% was 53% (95% CI: 41–70). For plasma iohexol clearance studies, the mean GFR measured by inulin was  $66 \pm 40$  mL/min/1.73 m<sup>2</sup>. Median bias for iohexol was  $+3\%$  (95% CI: 0–6). Pooled accuracy within 30% was 86% (95% CI: 81–91); accuracy within 10% was 50% (95% CI: 43–58). We agree with the conclusions of the authors of this analysis: urinary clearance of iohexol is sufficiently accurate to reflect inulin clearance but the evidence is limited (47 measurements in 47 participants); plasma clearance of iohexol is also sufficiently accurate with moderately strong evidence (172 measurements in 172 participants) [3].

### Iohexol: studies comparing iohexol with other reference markers

We will focus on studies with the most appropriate methodology, i.e. studies with low injected doses (usually no more than 5 or 10 cm<sup>3</sup> of iohexol 300 or 240 mg I/mL) but also with the same procedure used for any markers (Table 3). Indeed, as will be discussed in the next paragraph, different procedures (urinary versus plasma clearance or different sampling times) potentially lead to differences in GFR results, which makes it impossible to know whether these differences are due to the marker itself or to the methodology. Finally, we emphasized results from studies that included appropriate statistical tools, such as Bland and Altman or accuracy analyses.

<sup>51</sup>Cr-EDTA clearance is one of the most accurate methods to measure GFR [3, 116, 117]. It is thus not surprising that several authors have studied the concordance between both <sup>51</sup>Cr-EDTA clearance and iohexol clearance [22, 30, 58, 68, 69, 72, 84, 92, 93, 109, 113–115]. All relevant studies compared plasma clearances of both markers and have found excellent agreement [30, 69, 72, 92, 93, 109, 115]. Among these studies, five authors provided Bland-Altman analyses, displaying little bias (between 0 and 4 mL/min) and excellent precision (95% of the results between 8 and 16 mL/min/1.73 m<sup>2</sup>) [58, 69, 72, 93, 115]. Based on these results, one can conclude that plasma clearance of iohexol is concordant with plasma clearance of <sup>51</sup>Cr-EDTA.

Only a few studies compared <sup>99</sup>Tc-DTPA with iohexol and the majority have questionable methodologies [66, 68, 70, 102, 111]. A study in 21 diabetic patients showed good correlation, acceptable bias (4 mL/min) and precision for plasma clearance of iohexol and <sup>99</sup>Tc-DTPA [70].

Studies comparing iohexol and iothalamate clearances are of importance as iothalamate, an ionic contrast medium, is the most frequently used GFR marker in the USA and the only 'cold' alternative method to iohexol for measuring GFR. At least five studies have compared the two markers [24, 26, 32, 82, 108]. Two analyses, performed in healthy people, showed a constant bias in plasma clearances, with iothalamate clearances being systematically higher than iohexol clearances [26, 108]. A third one included patients with severe CKD but results were unreliable because statistical analyses were not adequate [24]. In a recent study, the authors compared urinary clearances of iohexol and iothalamate in 150 kidney transplant recipients. Contrary to the majority of other studies, iohexol was measured by LC-MS/MS. The authors found iothalamate urinary clearance to be systematically higher (+15%, constant bias thorough the GFR range) than iohexol urinary clearance [32]. Finally, another recent study compared plasma clearance of iohexol and iothalamate in 102 patients with a wide range of GFR. The authors found a good concordance between iothalamate and iohexol plasma clearance measured by HPLC, whereas iothalamate systematically

Table 2. Studies comparing iohexol and inulin clearance

Reference	Sample size	Population	GFR range (mL/min/1.73 m <sup>2</sup> )	Methodology	Statistics	Results	Comments
Lewis [102]	29	Heart transplanted (n = 10) Kidney transplanted (n = 11) Living kidney donor (n = 10)	10–117	In UC Io (XRF) PC: samples at 180 and 240 min	Correlation Regression Ratio	0.86 Io = 0.85In + 8.79 1.09 ± 0.06	High dose of iohexol Ratio at 1.36 for 6 patients with GFR <20 mL/min BA (Io – In) calculated: 4.3 ± 27 mL/min Correction for the first curve NA (if any)
Brown [100]	30	Post-surgery	10–125	In UC Io (XRF) UC (three collections of 30 min) PC: samples at 180 and 240 min + BM correction	Correlation Regression Ratio	UC 0.986 Io = 0.998In – 2.31 PC 0.983 Io = 0.947In + 4.92 1.102 ± 0.286	One patient with oedema High dose of iohexol (10–50 cm <sub>3</sub> )
Lindblad [103]	46	Children	25–150	In UC (n = 54) PC (n = 20) Io (XRF) PC: samples at 60, 120 and 180 min	Correlation	0.766	Different doses injected Correction for the first curve NA Correlation at 0.822 if one nephrotic patient excluded
Gaspari [52]	41	CKD	6–160	In UC Iohexol (HPLC) PC: samples at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 450 and 600 min AUC samples at 120 and 600 min + BM correction	Correlation Regression BA In – Io	AUC 0.97 Io = 0.994In + 2.339 1.02 ± 7	4 had moderate oedema Regression not different from identity line Results in 20 patients with GFR <40 (BM): r = 0.908 Io = 0.852In + 4.789
Erley [104]	31	Intensive care unit	10–130	In UC Io (XRF) PC: samples at 150, 195, 240 and 360 min (360 if estimated GFR below <30 mL/min)	Correlation Regression BA Io – In	0.98 Io = 0.971In + 7.65 =8.67 ± 7.21	Authors do not mention how PC was calculated 9 patients had iopromide clearance instead of iohexol
Sterner [101]	20	Healthy	106–129	In UC Io (HPLC) UC Infusion, plasma samples at 270, 330, 390 and 420 min and urinary collection between 300 and 360 and 360 and 420 min (bladder scan)	Wilcoxon	In 117 mL/min Io 113 mL/min	No significant difference

Berg [105]	60	CKD children	5–180	In UC Io (HPLC)	Inulin 0.921	Correlation Regression BA (PC – In) Accuracy within 30%	Mean GFR are not significantly different
				PC: samples at 180, 200, 220, 240, 420 and 1440 min (420 if estimated GFR between 20 and 50 mL/min and 420 and 1440 if estimated GFR <20 mL/min) + BM correction	PC = 0.9In + 9.72 2.65 ± 16.26 83.3% Iohexol PC 0.659.6		Intraclass coefficient of correlation: 0.92

GFR, glomerular filtration rate; AUC, area under the curve; BA, Bland and Altman (bias ± standard deviation). BA calculated means that BA results have recalculated from data available in the article. BM, Brochner-Mortensen; CKD, chronic kidney disease; HPLC, high performance liquid chromatography; In, inulin; Io, iohexol; NA, not available; PC, plasma clearance; UC, urinary clearance; XRF, X-ray fluorescence. All GFR results are in mL/min or in mL/min/1.73 m<sup>2</sup>.

overestimated (by 10%) iohexol results measured by mass spectrometry [82].

Generally, these results head towards the same direction: GFR measured with iohexol yields higher results than iohexol. According to Seegmiller *et al.*, there are different theoretical explanations for this discrepancy: iohexol tubular reabsorption, iohexol tubular secretion or iohexol binding protein [32]. However, among these hypotheses, only the tubular secretion of iohexol is well described in the literature [42, 43]. Our interpretation is that iohexol is a better marker of GFR than iohexol because tubular secretion of iohexol leads to GFR overestimation.

### Plasma clearance: between physiology, safety and pragmatism

A variety of methodologies or procedures can be used to measure GFR with iohexol; most of these are not iohexol-specific and can be applied to other markers like <sup>51</sup>Cr-EDTA or iohexol. Different procedures have different degrees of complexity and precision and can therefore lead to slightly different results.

### Urinary versus plasma clearance

Iohexol is almost always injected as a bolus either for urinary or plasma clearance determinations, whereas constant infusion of iohexol [30, 36, 101] for urinary clearance measurement has seldom been used (Supplementary data, Table S1). Several publications have compared iohexol urinary and plasma clearances [28, 33, 36, 48, 77, 100, 101, 118]. Among these studies, the results published by Stolz *et al.* should be highlighted because 342 subjects were included and adequate statistical tools were applied [77]. Most studies find plasma clearances to be higher than urinary clearances. Two important points must be emphasized.

First, the timing of the last plasma sample in multiple-sample methods is fundamental: concordance between urinary and plasma clearance improves if the last iohexol measurement was performed late. This is particularly true in the very low GFR ranges [77, 118, 119]. Therefore, in all CKD patients (i.e. GFR <45 mL/min), it should be recommended to extend plasma sampling until 5, 6 or even 8 h after injection. Gaspari *et al.* studied GFR in a cohort of patients with CKD (GFR <40 mL/min) and plasma samples taken hourly up to 8 h. GFR was calculated by using all available samples or by using only those collected up to 5 h after iohexol injection. The authors showed that GFR was overestimated on average by 7% in comparison with GFR measured up to 8 h (F.G., personal data). Thus, timing of the last sample is crucial. In more advanced CKD (stage 5), an additional sample after 24 h has been recommended by some groups [48, 77]. This late sample is however not always easy to implement in clinical practice. This approach has been questioned by others arguing for the circadian variation of GFR [85, 87, 120]. Moreover, a rather long interval between the two last samples could potentially make the mathematical weight of the 24 h sample exaggerated in the final GFR calculation.

Second, urinary clearance is the only option in specific clinical situations where marker distribution takes several days, i.e. in patients with severe oedema or ascites.

Both of these two last requirements apply to all plasma clearance procedures and to all GFR markers [4, 107, 121–125].

We recommend a pragmatic approach when choosing the GFR measurement method: while urinary clearance may be the most physiologic and accurate method to measure the filtering capacity of the kidney, it is time consuming and prone to errors

Table 3. Studies comparing iohexol clearance with other GFR markers (except inulin)

Reference	Sample size	Population	GFR range (mL/min/ 1.73 m <sup>2</sup> )	Methodology	Statistics	Results	Comments
Olsson [22]	10	Healthy	NA	Cr UC Collection between 120 and 240 and between 240 and 360 min Io (HPLC) UC idem		UC 120–240 min Io: 112 mL/min Cr: 95 mL/min 240–360 min Io: 109 mL/min Cr: 97 mL/min	High doses of iohexol
Krutzen [30]	42	Healthy and CKD	24–207	Cr PC: 120 and 240 min + BM correction Io (HPLC) idem	Correlation Regression	0.983 Io = 0.99Cr – 1.92	
O'Reilly [84]	54 (100 cm <sub>3</sub> Io) 33 (50 cm <sub>3</sub> Io)	NA	30–130	Cr PC: samples at 60, 120, 180 and 240 min Io (XRF) PC: samples at 180 and 240 min	Correlation	100 cm <sub>3</sub> : 0.9 50 cm <sub>3</sub> : 0.85	High doses of iohexol Correction for the first curve NA
Bäck [108]	18	Healthy	NA	It (HPLC) PC: samples at 160, 180, 200, 220, 240 and 260 min Io (HPLC) idem		Mean GFR by It 40% higher than mean GFR by Io	Different doses are injected (2 to 20 cm <sub>3</sub> )
Bäck [26]	7	Healthy women	100–140	It (HPLC) PC: samples at 2, 5, then every 5 min for 90 min then 120, 180, 200 and 220 min AUC Io (HPLC) idem		Io: 121 mL/min It: 144 mL/min	
O'Reilly [66]	33 measurements in 12 subjects	NA	20–100	Dt PC: samples at 90, 135, 180 and 240 min Io (XRF) PC: samples at 180 and 240 min	Correlation	0.95	High doses of iohexol Correction for the first curve NA
Lewis [102]	29	Heart transplanted (n = 10) Kidney transplanted (n = 11) Living kidney donor (n = 10)	10–117	Dt UC: 3 collections of 20 min Io (XRF) PC: samples at 180 and 240 min	Correlation Regression Ratio	0.89 Io = 0.89Dt + 6.5 1.08 ± 0.06	High dose of iohexol BA calculated: –0.7 ± 14.8 Correction for the first curve NA
Effersöe [68]	15	Patients for urography	22–110	Cr and Dt PC: samples at 0, 10, 20, 30, 120, 180, 240 and 300 min AUC Io (XRF) idem	Regression Correlation BA Cr – Io Dt – Io	Io = 0.97Dt – 11 0.96 Io = 1.01Cr + 8 0.95 –10.8 ± 7.9 –9.4 ± 6.9	High doses of iohexol

Eriksson [109]	98	Diabetics	30–150	Cr PC: samples at 220 min + Jacobsson correction [110] Io (HPLC) idem	Regression Correlation	Cr = $1I_o - 0.8$ 0.965	
Stake [111]	11	Children with severe CKD	8–30	Dt PC: samples at 5, 15, 120, 150, 180 and 210 min + Rootwelt correction [112] Io (XRF) PC: 6 samples between 180 and 1440 min + BM correction	BA Dt – Io	Io 6 samples: $13 \pm 2$ mL/min Dt: $17 \pm 2$ mL/min	High doses of iohexol Dt significantly higher than Io 6 samples but no difference if Io sampled at the same time as Dt
Lundqvist [113]	31	Plegic patients	70–130	Cr PC: samples at 180, 210, 240 and 270 min + BM correction Io (XRF) idem	BA Cr – Io	Day 1: $+2.1 \pm 10.2$ Day 2: $+0.9 \pm 5.9$	High doses of iohexol
Nossen [24]	8	Severe CKD	5–9	It UC: 11 collections between 0 and 7200 min PC: samples at 30, 60, 120, 180, 360, 540, 1440, 1920, 2880, 4320 and 7200 min AUC Or BM correction Io (XRF) idem	Mean	UC It $6 \pm 2$ Io $6 \pm 2$ PC: AUC It $6 \pm 2$ Io $8 \pm 1$ PC: BM Io $8 \pm 1$	High doses of iohexol
Rydström [92]	122	Healthy and CKD	4–139	Cr PC: samples at 180, 200, 220, 240 and 1440 min (1440 min if creatinine > 2 mg/dL) + BM correction Io (XRF) idem	Correlation Regression	0.986 $I_o = 0.971Cr - 1.368$	
Lundqvist [114]	77	Patients for urography	25–125	Cr PC: samples at 180, 210, 240, 270 min + BM correction Io (XRF) PC: samples at 180 and 240 or 270 min + BM correction	Correlation Regression BA (Io – Cr)	0.918 $I_o = 0.892Cr + 6.278$ $2 \pm 9.2$	High doses of iohexol

Table continues



Table 3. Continued

Reference	Sample size	Population	GFR range (mL/min/ 1.73 m <sup>2</sup> )	Methodology	Statistics	Results	Comments
Brandstrom [69]	49	CKD patients GFR >40 mL/min	40–125	Cr PC: samples at 150, 195 and 240 min + BM correction Io (HPLC and XRF) idem	Regression Correlation BA Cr – Io	XRF Io = 1.03Cr – 1.79 0.97 0.58 ± 4.95 HPLC Io = 1.05Cr – 4.43 0.96 –0.16 ± 6.17	Slope not different from 1 and intercept not different from 0
Pucci [72]	32	Diabetics	13–151	Cr PC: samples at 5, 10, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420 and 1440 (360 and 420 if creatinine >2 mg/dL, 1440 if creatinine >5 mg/dL) AUC Io (HPLC) idem	Regression Correlation BA Cr – Io	Io = 0.978Cr + 2.45 0.995 –0.6 ± 3.6	
Houlihan [70]	21	Diabetics	50–145	Dt PC: samples at 120, 165 and 210 min + BM correction Io (XRF) PC: samples at 120, 150, 180, 210 and 240 min + BM correction	Regression Correlation BA Io – Dt	Io = 0.9938Dt + 4.916 0.97 4.3 ± 7.7	
Pucci [93]	41	Diabetics	29–150	Cr PC: samples at 5, 10, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420 and 1440 (360 and 420 if creatinine >2 mg/dL, 1440 if creatinine >5 mg/dL) AUC Io (HPLC) idem	Regression Correlation BA Cr – Io	Type 1 Io = 0.978Cr + 0.132 0.999 Type 2 0.987 Io = 0.078Cr + 2.352 –0.42 ± 3.69	
Bird [115]	56 19	Patients (diabetes, cancer) Healthy, studied two times (fasting or not)	15–140	Cr PC: samples at 20, 40, 60, 120, 180 and 240 min AUC Io (XRF) idem	BA Cr – Io	4 ± 7.9	
Slack [58]	10	Cirrhotic	45–100	Cr PC: samples at 120, 180 and 240 min +BM correction Io (HPLC) idem	BA Cr – Io	–1.3 ± 8.6	

Seegmiller [32]	150	Healthy and CKD	5–150	It U: one collection over 45 or 60 min after 60 min + bladder scan Io (mass spectrometry)	Passing Bablok BA relative % Io – It	Io = 0.85It + 0.44 –15% ± 10%	Slope different from 1 but intercept not different from 0
Delanaye [82]	102	Healthy and CKD	15–130	It PC: samples at 120, 180, 240 and 300 min + BM correction Io (HPLC and mass spectrometry) idem	HPLC Passing Bablok BA relative % Io – It Accuracy within 15% Mass spectrometry Passing Bablok BA relative % Io – It Accuracy within 15%	Io = 0.88It + 7 –2% ± 13% 80% Io = 0.88It + 1 –11% ± 9% 74%	

GFR, glomerular filtration rate; AUC, area under the curve; BA, Bland and Altman (bias ± standard deviation). BA calculated means that BA results have recalculated from data available in the article. BM, Brochner-Mortensen; CKD, chronic kidney disease; Cr, <sup>51</sup>Cr-EDTA; Dt, <sup>99m</sup>Tc-DTPA; HPLC, high performance liquid chromatography; Io, iohexol; It, iothalamate; NA, not available; PC, plasma clearance; UC, urinary clearance; XRF, X-ray fluorescence. All GFR results are in mL/min or in mL/min/1.73 m<sup>2</sup>.

in the measurement of urinary flows. Plasma clearance methods represent the best compromise between physiology and feasibility, both in clinical routine and research [for example: REIN (Ramipril Efficacy In Nephropathy), REIN-2, DEMAND (Developing Education on Microalbuminuria for Awareness of Renal and Cardiovascular Risk in Diabetes) and ALADIN (A Long-Acting Somatostatin on Disease Progression in Nephropathy due to Autosomal Dominant Polycystic Kidney Disease) studies] [126–129]. Monitoring plasma clearance is far less cumbersome and costly, especially in older adults or diabetic patients with bladder dysfunction and young children for whom urine collection remains challenging [130, 131]. Once again, these limitations are common in all GFR markers, including the gold standard inulin. Bladder catheterization has been used in small studies but this approach is obviously not feasible in clinical trials or clinical practice [132].

There may be one potential limitation of urinary clearance that applies specifically to iohexol, as some authors have actually hypothesized that measurement of iohexol in urine could be problematic (matrix effect). This matrix effect remains however purely speculative and other authors do not have any problem in quantification of iohexol in urine (F.G., personal data). This point is thus the subject of debate and would need further studies [118].

#### Plasma clearance: ok but which one?

Because of its physical characteristics (viscosity and density), iohexol is particularly suitable for plasma clearance measurement. After a bolus injection, plasma iohexol concentration will decrease constantly according to two different exponential curves (see Figure 1). The first rapid curve (fast component) corresponds to the volume of distribution, i.e. the extracellular volume [22–26]. The second curve (slow component) corresponds to the clearance of the marker by the kidney. There are several different methodologies to calculate GFR from the disappearance curves. The most precise method is to measure iohexol in both components, and to calculate the area under the curve which eventually allows GFR calculation [which corresponds to the dose of iohexol injected divided by the area under the curve (AUC)]. This method, named AUC in the current article, implies sampling of several plasma measurements at different time points. Ioexol should be sampled at very short intervals at the beginning (at 5, 10, 20, 30, 45, 60, 90 and 120 min after injection [52]), and thereafter every hour. This methodology is complex and costly, especially with necessary repeated samples during the first 2 h. For this reason,

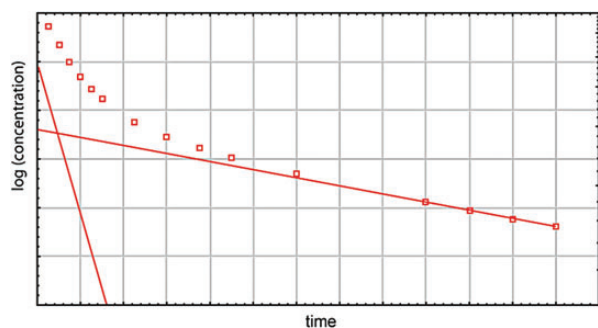


Fig. 1. Elimination of iohexol from plasma after a single injection. Following a single injection, iohexol concentration in plasma falls as a result of both distribution and elimination. In a semi-logarithmic plot these phases can be illustrated by two lines, the slopes of which are proportional to the half-life of each phase.

authors have proposed a mathematical correction for the first fast exponential curve. This method is frequently named as the slope-intercept method. Different mathematical corrections have been proposed [131, 133, 134, 135]. The impact of these different corrections on GFR results is probably relatively limited. The correction proposed by Brochner-Mortensen (BM) [116], initially developed for  $^{51}\text{Cr}$ -EDTA, is the most used in Europe:

BM correction =  $0.990778 \times C_2 - 0.001218 \times C_2^2$  (where  $C_2$  is the GFR calculated on the second curve only) [116]. The correction proposed by Ng et al. both for adults and children [131] has still been poorly externally validated but has been developed from a state of the art methodology and deserves further study [118, 131].

The most important limitation of the mathematical correction of the first curve area is the trend to underestimate high GFR levels (which could lead to underdiagnosis of renal hyperfiltration) [136, 137].

Different protocols have been proposed to calculate the area under the second curve (the curve that impacts the GFR result). The number and, even more important, the timing of samples are fundamental. A simple procedure would be to measure iohexol at two or three different time points. Restricting the GFR measurement to two points might theoretically expose to a greater risk of errors (if the first sample is drawn too early or the last sample not late enough). However, most studies with this protocol show that this easy-to-use procedure gives accurate and concordant results with urinary clearances or multiple-samples plasma clearances [52, 135]. When using simplified protocols, it is recommended to perform the first sample not earlier than 2 h after injection. The choice of the timing for the last sample depends on expected GFR levels [48, 52, 130]. For example, in patients with normal GFR values, the last measurement can be sampled 4 h after the injection because later samples would lead to the risk of complete clearance of iohexol. In CKD patients, the precision of the GFR calculation will be higher if iohexol is sampled later, i.e. after 5, 6 or 8 h or even after 24 h in pre-dialysis patients. Supplementary data, Table S2 summarizes studies on the agreement between different plasma clearance procedures.

Eventually, an even more simplified procedure with only one sample of iohexol can be used to calculate GFR. Different mathematical procedures have been proposed for the calculation of this single-sample method [92, 110, 114, 138–148]. The equation proposed by Jacobsson is certainly the most popular in Europe [110], and, for some investigators, the most precise method [113, 114].

$$\text{GFR} = (1/(t/V + 0.0016)) \times \ln(\text{Dose}/(V \times C_t)) \text{ (in mL/min)}$$

$$V_{\text{male}}: 166 \times W + 2490$$

$$V_{\text{female}}: 95 \times W + 6170 \text{ [110]}$$

$C_t$  is the sample concentration ( $\mu\text{g/mL}$ ) at time  $t$  (minute),  $V$  is the apparent volume of distribution (mL) and  $W$  is the weight (kg).

Determination of iohexol by the single-sample method obviously has great practical advantages. Again, the timing of the single-sample is important as it impacts the precision of the GFR calculation. As previously described, the best results are obtained when the timing of the sample is adapted to the expected GFR (based on the subjects estimated GFR), which is however sometimes difficult in very specific patients like hyperfiltrating subjects. An optimal sampling time to minimize the uncertainty in the estimate of the volume of distribution can be calculated from a formula derived by Jacobsson [110]. The sample should be late if GFR is low (300–360 min if GFR between 30 and 60 mL/min and 600 or 1440 min if GFR is below 30 mL/min) and early (180 min) if GFR is normal or high [105, 115, 149, 150]. Since weight is part of the equation, the Jacobsson correction might be

questionable in subjects with extreme weights. Indeed, the estimation of extracellular volume is one predominant source of error with the single-sample method. Also, the single-sample method requires a very high precision of iohexol determination in plasma, which is why some authors have suggested that HPLC-UV be favoured over XRF with this procedure [69, 150]. In all plasma procedures, potential random analytical, blood drawing or timing errors can sometimes occur, but if there is any error, potentially outlier points can be easily identified, observing the declining curve built from multiple-samples results. Outlier points can thus be discarded (or measurement be repeated). This sort of internal control does not exist with the single-sample method. In Supplementary data, Table S3, we have summarized the available data for single-sample iohexol plasma clearance [24, 33, 69, 92, 98–101, 103, 105, 109, 114, 115, 149–154]. In general, the method demonstrated good agreement with either urinary inulin or plasma <sup>51</sup>Cr-EDTA clearance. No study has appropriately compared the performance of single- and multiple-sample methods with inulin urinary clearance as the gold standard reference. Bird *et al.* found the performance of the single-sample to be at least as good as that of the multiple-sample method for normal range GFR when compared to multiple-sample <sup>51</sup>Cr-EDTA clearance [115]. Several authors have compared concordance between single-sample and multiple-sample procedures with iohexol. Strictly speaking, such studies do not allow the performance of the two procedures to be tested, as the methods are not independent of each other, but the results of concordance between the two procedures are still of interest. Among these studies, the publication of Gaspari *et al.* is certainly the most relevant, including 686 patients. These authors found a slight overestimation of 3.2 mL/min/1.73 m<sup>2</sup> of the single-sample method for GFR over 100 mL/min/1.73 m<sup>2</sup> when comparing it with the

multiple-sample method, but after logarithmic transformation the difference was the same as when using the optimal sampling time for GFR between 40 and 49 mL/min/1.73 m<sup>2</sup> [150]. However, if bias is acceptable, concordance is not always perfect as 75% of results are concordant, concordance being defined as results within 5%. Another definition of concordance (within 10%) would lead to still more acceptable concordance, especially, once again, if the sample timing is adapted to the expected GFR.

Single-sample plasma clearance could also be used to measure residual renal function in haemodialysis patients (iohexol is injected at the end of one dialysis session and a plasma sample is captured at the start of the next treatment) [35].

Once again, all these considerations about timing and number of samples are not iohexol-specific, but also apply to other GFR markers, such as <sup>51</sup>Cr-EDTA or iothalamate [117, 142, 143, 155–167].

### A pragmatic proposal

The choice of the procedure (urinary or plasma clearance, timing and number of plasma samples) will depend on human and financial resources and specific centre expertise/experience. The choice of the procedure may depend on the reason (or the context) of why GFR is being measured. The single-sample plasma clearance technique is probably the more cost-effective procedure for population studies and renal function monitoring in large cohorts of patients. If feasible, the multiple-sample plasma clearance method is probably the most effective approach to monitor intra-patient GFR changes over time in the context of clinical trials and every-day clinical practice in individual patients. In Table 4, we make some suggestions for the choice of procedures according to the context of GFR measurement. This

**Table 4.** Available procedures to perform iohexol clearance

Methodology	Indication in clinical practice	Indication in clinical research	Bibliographic examples where the procedure is described into details
<i>Urinary clearance</i>	Increased extracellular volume (oedema, ascites, intensive care units, etc.)	Basic (physiologic) studies Specific populations (cirrhotic, intensive care, nephrotic syndrome, oedema, etc.)	[36, 77, 125, 170]
<i>Plasma clearance</i>			
Multiple samples (first or fast, second or slow exponential curves and calculation of area under the curve)	High GFR values ("hyperfiltrating") subjects	Development of equations to estimate GFR Studies in hyperfiltrating patients	[52, 93, 171]
Multiple samples only for second and slow component (2 h after injection, 4 samples over 5 or 6 h, 1 sample/h) + BM correction	High precision determination (see text)	Development of equations to estimate GFR Clinical research with GFR as main endpoint	[126, 172]
Idem + late sample (8 h or 24 h)	Pre-dialysis subjects	Research in pre-dialysis subjects	[52, 77]
Simplified two or three sample method (2 samples: first at 2 or 3 h and second at 4 or 5 h) + BM correction	CKD or healthy population	Development of equations to estimate GFR Clinical research with GFR as a secondary endpoint	[69, 116]
Simplified single-sample method + Jacobsson correction [110]	CKD or healthy population	Development of equations to estimate GFR Clinical research with GFR as a secondary endpoint Epidemiological research	[14, 173]

Suggestions (expert opinion-based) according to the clinical or experimental context. GFR, glomerular filtration rate; CKD, chronic kidney disease; BM, Brochner-Mortensen correction [116].

table represents a consensus based on the opinion of the authors of the current article.

Any of these methods, including the simplest procedure, can be considered as a good GFR measurement and may be considered a better reflection of 'true' GFR than any equation based on serum creatinine and/or cystatin C, as these biomarkers are influenced by non-GFR determinants.

### The fact: we need standardization for measuring GFR

The fact that numerous different markers exist to measure GFR and that, for each marker, different protocols have been described, explains at least in part, why measured GFR has been questioned [174, 175]. Standardization is a necessary step in promoting GFR measurement. However, standardization is not easy and can be lengthy. For example, it took years to standardize the measurement of a simple biological parameter like serum creatinine [176]. For measured GFR, we need standardization at three different levels. First, the measurement of the markers in plasma must be standardized. As already mentioned, this is actually not the case for markers like inulin and iothalamate. Today, measurement of iohexol by HPLC-UV is the most popular and probably the best balance between sensitivity-specificity on one side and accessibility-cost on the other. In the analytical context, the presence of a proficiency programme (external quality control) and the stability of iohexol are two key advantages for this marker. Second, we need standardization regarding the marker used. We do not question the accuracy of isotopic methods to measure GFR, but, by nature, these methods are difficult to implement wherefore they remain limited to nuclear medicine units. Iothalamate clearance overestimates real GFR because of tubular secretion. Another major issue with iothalamate is its lack of availability, notably in Europe. Inulin is expensive and can only be used with urinary protocols. Iohexol plasma clearance seems to be the best choice considering availability, cost, safety and feasibility. Third, the procedure to measure GFR should be standardized and we have made some pragmatic propositions earlier in the text (Table 4).

### Conclusions

'Measuring GFR is cumbersome and costly' is a sentence that has been written by numerous authors, including some of us, to justify the use of estimated GFR. It would be counterproductive to assert that measuring GFR is as simple as measuring creatinine or cystatin C. However, measured GFR is a reference method. Therefore, it should be compared to other reference methods in medicine. Troponin is a key biological parameter for cardiologists and we can compare it to serum creatinine for nephrologists. The sensitivity of troponin to detect myocardial infarction but also to predict mortality has however not questioned the relevance of coronary angiography as the reference method. The comparison might be continued, notably regarding the terms 'cumbersome' and 'costly'. As we described earlier, measuring GFR by iohexol plasma clearance can be simplified, while keeping a high degree of precision. Iohexol is available worldwide and provided under the commercial name Omnipaque® or Accupaque® (240 or 300 mg I/mL) in Europe. Different bottles of iohexol exist and the price of one bottle of 20 cm<sup>3</sup> (to be used for two or four patients, if 5 or 10 cm<sup>3</sup> are injected) is around 10 Euros in Belgium. Including the price of the HPLC measurement, and depending on

the number of samples, the global procedure will cost between 100 and 200 Euros per patient. This cost is comparable to the cost of other procedures in medicine like any CT scan. The safety of iohexol protocols is also remarkably superior to that of other reference methods in medicine such as colonoscopy or coronary angiography. According to the characteristics of iohexol, we think that iohexol plasma clearance is clearly the best alternative to have the same, standardized method for GFR measurement that would be easy to implement worldwide.

### Supplementary data

Supplementary data are available online at <http://ckj.oxfordjournals.org>.

### Conflict of interest statement

None declared.

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