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Accuracy of determination of the glomerular filtration marker iohexol by European laboratories as monitored by external quality assessment

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Abstract

Background: Glomerular filtration is the most important kidney function. The most accurate glomerular filtration rate (GFR) estimates are based on the clearance of exogenous filtration markers. Of these, iohexol is the only exogenous marker that is included in an external quality assessment (EQA) scheme. The aim of the present study was to evaluate the performance of the European laboratories participating in Equalis' EQA scheme for iohexol.

Methods: Weighed amounts of iohexol (Omnipaque) were added to plasma samples and distributed to laboratories participating in the EQA scheme for iohexol. All laboratories performed the assays in a blinded fashion.

Results: The number of participating laboratories varied between 27 and 34 during the study period. Iohexol was determined by HPLC in 77% of the laboratories and by UPLC/MS/MS methods in 15% of the laboratories. The mean interlaboratory coefficient of variation was 4.7% for the HPLC methods and 6.4% for the UPLC/MS/MS methods. The mean bias between calculated and measured iohexol values was -1.3 mg/L (95% confidence interval ± 0.3) during the first part of the study period and 0.1 mg/L (± 0.3) during the later part.

Conclusions: The low interlaboratory variation demonstrates that iohexol can be measured reliably by

many laboratories and supports the use of iohexol as a GFR marker when there is a need for high quality GFR measurements.

Keywords: external quality assessment scheme; GFR; glomerular filtration rate; iohexol.

Introduction

Glomerular filtration rate (GFR) measurements are essential for monitoring patients with suspected kidney diseases and for verifying normal kidney function in living kidney donors. The gold standard method for measuring GFR has been renal clearance of inulin measured during continuous infusion and urine collection [1]. This is a cumbersome method rarely used today, and inulin has been substituted with other exogenous filtration markers such as ^{99m}Tc -diethylenetriaminepentaacetic acid (^{99m}Tc -DTPA), chromium 51-labeled ethylenediaminetetraacetic acid (^{51}Cr -EDTA), iohexol and iothalamate [2]. Several of these markers are radiopharmaceuticals meaning they have short half-lives, expose the patient to radiation and are more expensive [3].

In contrast, iohexol is a non-ionic, non-radioactive X-ray contrast medium [4, 5], sold under brand names such as Omnipaque and Hexopaque [6]. Iohexol is also available as a density gradient medium under the names Nycodenz, Histodenz and Accudenz [7, 8]. For more than 30 years, iohexol has been used as an exogenous GFR marker. Furthermore, iohexol is considered as one of the safer exogenous GFR markers [9]. The amount of iohexol administered for GFR measurements is much smaller, typically 5 mL or less, than when used as a contrast medium. It is also sufficient to measure the plasma concentration of iohexol, eliminating cumbersome urine collections and simplifying the procedure.

Certified reference materials for iohexol or any other of the exogenous GFR markers are as yet unavailable. Instead, laboratories performing iohexol measurements use the registered contrast medium formulation for calibration. As this is highly concentrated, serial dilution in

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several steps is required to ensure relevant iohexol levels for calibration; a procedure that may introduce error. To verify that the assay is without bias, participation in external quality assessment (EQA) schemes is important. The only EQA scheme in Europe for iohexol GFR determination is managed by Equalis, a nonprofit EQA organization in Sweden. The aim of the present study was to evaluate the performance of the participating European laboratories in this EQA scheme.

Materials and methods

EQA material

The results of a total of 14 EQA rounds sent out from Equalis (Uppsala, Sweden) during the time period 2015–2018 were investigated. Each EQA round consisted of two samples. For preparation of the EQA materials, Omnipaque (300 mg I/mL, GE Healthcare Ireland, Cork, Ireland) was diluted (weight values) in pooled plasma from healthy blood donors to achieve calculated iohexol concentrations in the range of 20–105 mg/L. The absence of iohexol in the original plasma pool was verified by HPLC. The method was only used to verify that there was no iohexol in the samples prior to addition of the iohexol to the EQA samples. The values assigned to the EQA samples were either the consensus value reported by the participants or the weighed-in values. All dilutions and the HPLC measurements were performed at the Department of Clinical Chemistry (Karlstad, Sweden). The diluted preparations were carefully mixed to ensure even distribution of iohexol and sent to Equalis in liquid form. At Equalis the materials were pipetted into 2 mL tubes with 0.8 mL sample per tube and stored at -20°C until distribution to participating laboratories. Ethical approval for preparing EQA materials from surplus unidentified pooled blood donor plasma was not required.

Statistical analysis

Interlaboratory variation, linear correlations and deviation of mean values from calculated values were calculated using Excel 2013 (Microsoft, Seattle, WA, USA). Mean values and coefficient of variation (CV) were calculated according to the robust procedure described in the standard ISO 17043 (www.iso.org), as stated. The change in bias between the first seven EQA distributions and the seven most recent surveys was analyzed using Statistica 4.5 (StatSoft Inc, Tulsa, OK, USA).

Results

CV for the different EQA materials

The number of laboratories reporting test results varied between 27 and 34 during the study period. The majority

(77%) of the results were analyzed using HPLC, 15% used UPLC/MS/MS and the remainder (8%) used UPLC, HPLC/MS/MS or “unknown methods”. The interlaboratory CV for the different EQA materials varied between 2.7% and 7.1% (mean 4.7%) for the HPLC methods, and between 1.5% and 12.2% (mean 6.4%) for the UPLC/MS/MS methods (Table 1 and Figure 1).

The interlaboratory CV was slightly higher in materials with iohexol levels around 20 mg/L than in higher concentrations (Figure 1). The interlaboratory CV for the UPLC/MS/MS methods was generally somewhat higher than for the HPLC methods (Figure 2).

Table 1: Interlaboratory CV for samples sent out in each external quality assessment round.

Material	n	Iohexol, mg/L		Reproducibility CV	
		Mean	Robust mean	CV, %	Robust CV, %
2015:01/A	30	116.2	116.3	4.59	4.65
2015:01/D	27	63.0	63.0	4.00	3.73
2015:02/D	29	64.2	64.2	4.29	4.85
2015:02/B	28	22.9	22.8	6.55	6.65
2015:03/E	29	81.1	81.0	4.14	4.32
2015:03/C	29	43.2	43.1	4.37	4.55
2015:04/F	32	102.1	101.7	5.81	5.72
2015:04/D	32	64.0	63.9	5.85	4.76
2016:01/C	32	43.7	43.8	5.35	5.93
2016:01/B	32	22.3	22.3	6.54	7.09
2016:02/F	29	100.9	100.9	4.12	4.43
2016:02/C	29	43.7	43.8	4.49	4.53
2016:03/E ^a	29	82.5	82.3	3.61	3.62
2016:03/C	30	44.1	44.0	3.82	3.64
2016:04/C	33	44.8	44.7	4.68	3.57
2016:04/B	33	22.8	22.8	6.12	5.64
2017:01/F	31	103.7	103.1	6.76	6.08
2017:01/E	32	83.8	83.4	7.03	6.11
2017:02/I	30	55.6	55.3	5.24	4.52
2017:02/G	31	20.1	20.0	6.10	5.34
2017:03/K	30	99.6	99.5	5.04	4.81
2017:03/H	30	38.2	38.2	5.71	5.13
2017:04/J	30	85.4	85.3	3.84	4.18
2017:04/G ^a	30	20.2	20.2	6.89	6.80
2018:01/I	34	54.1	54.4	5.00	3.34
2018:01/H	33	37.7	38.0	6.37	3.95
2018:02/J	33	84.9	84.6	4.64	3.83
2018:02/H	33	38.1	38.1	3.72	4.12

^aOne extreme value excluded. In total 10 different materials (A–J) have been used during the period. Each round consisted of two samples. Mean values and CV calculated without exclusion of possible outliers and mean and CV calculated by robust methods are presented for each sample.

Figure 1: Mean interlaboratory CV (y-axis) vs. measured concentration of iohexol (x-axis) for laboratories using either HPLC or UPLC/MS/MS.

Figure 2: Between laboratory reproducibility for HPLC methods and UPLC/MS/MS methods vs. time of distribution. Since 2017, the reproducibility for HPLC methods has been better than for UPLC methods.

Within- and between-laboratory variation

Material C was used in five surveys and material H in three surveys. The data from these materials were used to compare within laboratory with between laboratory CV. The results showed that within laboratory CVs were larger than between laboratory variation (Table 2).

Bias between calculated and measured iohexol values

For materials B–F, distributed during the period 2015–2016, the mean bias was -1.3 mg/L (95% confidence interval ± 0.3), as compared to 0.1 mg/L (± 0.3) for materials G–J distributed during the period 2017–2018 ($p < 0.001$) (Figure 3).

Table 2: Eleven different materials have been used during the study period and distributed in 14 surveys.

Material	Weighed value, mg/L	Number of surveys	Total number of results	Measured mean value, mg/L	Within-lab CV	Between-lab CV
C	44.86	5	181	43.94	3.87	2.69
H	37.79	3	122	37.98	4.53	2.87

Material C (five surveys) and Material H (three surveys) were used to calculate within laboratory and between laboratory CV.

Figure 3: Bias between mean of measured iohexol concentrations and the iohexol value calculated when preparing the EQA materials. Error bars indicate 95% confidence intervals.

Discussion

GFR estimates have been made increasingly available over the last few decades. The interest can in part be traced back to the K/DOQI initiative [10] by the National Kidney Foundation, where laboratories were encouraged to provide a calculated GFR whenever a plasma creatinine was ordered; an initiative that spawned a plethora of GFR estimation formulas. An *estimated* GFR based upon plasma marker level in addition to a few anthropometric variables is often sufficient for daily clinical decision-making, but when the need for accuracy and precision is increased (kidney donation, treatment with nephrotoxic drugs, etc.) a *measured* GFR calculated from the plasma disappearance curve of an exogenous marker, e.g. iohexol, may be called for [4]. Iohexol has been used for this purpose for more than 30 years and has gained wide-spread recognition as a true

gold standard for GFR measurement. Iohexol can be analyzed in plasma after administration of a small dose, and as it is non-radioactive, stable in plasma and comparatively easily quantitated using HPLC or mass spectrometry, reliable monitoring of iohexol elimination is possible. However, continuous provision of high quality GFR measurements requires the analytical process to be strictly controlled. Participation in EQA schemes ensures that both bias and precision are checked on a regular basis, and participation is also a prerequisite for the analysis to be accredited according to ISO15189. Equalis’ iohexol EQA scheme is to the best of our knowledge the only available EQA scheme for exogenous GFR markers. The results in this study represent laboratories performing measurements of iohexol on a regular basis, mainly in Europe, 55% from Sweden and 40% from the rest of Europe. The remaining 5% are participants from Africa, Asia and the US.

To the best of our knowledge almost all participants use Omnipaque® as standard for the iohexol measurements. Occasionally, alternative modes of calibration have been reported, with acceptable results if the ratio between the two stereoisomers has been considered and is identical with that for the substance provided to the patient. Considering the lack of certified reference materials for iohexol, the preparation of each laboratory's own calibrators and the existence of several suppliers of iohexol, the mean interlaboratory CV found in this study can be considered low. The mean bias between calculated and measured iohexol values was also low, demonstrating a lack of calibration issues. In addition, bias was considerably lower in the latter seven rounds, suggesting that the participants have acted on their EQA results to review their assay method to further improve performance.

In principle, different sources of iohexol standards contribute to the interlaboratory bias. This will be further investigated in future studies.

The average levels of iohexol measured with HPLC and UPLC/MS/MS did not differ, while the interlaboratory CV was somewhat higher for the UPLC/MS/MS methods than for the HPLC methods (Figure 2). Thus, the discrepancy in measurement level between HPLC and LC-MS/MS methods noted by Delanaye and coworkers [11] is not confirmed by the data in this EQA scheme.

Iohexol determinations are exclusively used for measuring glomerular filtration rate; thus, the demands for analytical accuracy will be defined by the intra-individual variation in GFR as such. As GFR cannot be studied directly, arriving at a figure entails subtracting the analytical variation from the total variation as determined by repeated measurements of GFR in the same subject. By doing so, Krutzen and coworkers found a total variation of 11%, which given the analytical variation of 5% suggested an intra-individual variation in GFR of approximately 10% [12]. The subjects were healthy volunteers with GFR in the normal range. Other workers [5, 13, 14] have found similar or lower figures. However, none of the studies have fulfilled the Biological Variation Data Critical Appraisal Checklist (BIVAC) [15]. Despite the limitations of data, biological variation in GFR appears to be at least as high as the methodological imprecision for iohexol, which suggests that the latter does not contribute significantly to the total error of GFR measurement.

In conclusion, the EQA results demonstrate that iohexol can be measured reliably by the participating laboratories and further support the use of iohexol as a reliable GFR marker.

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