Development of an outpatient finger-prick glomerular filtration rate procedure suitable for epidemiological studies

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Development of an outpatient finger-prick glomerular filtration rate (GFR) procedure suitable for epidemiological studies. In clinical practice, reference GFR procedures are rarely used; in large-scale research studies, a great deal of effort and experience is required to obtain them, which is a considerable disincentive to using GFR as an end point. The major problem for both clinical staff and the subject is the length of time that the procedure takes, requiring continuous attendance in the outpatient clinic or its vicinity. Using iohexol as a marker, we therefore propose an alternative approach, which addresses this fundamental deterrent to a more widespread use of GFR measurement. Eighty-two GFR measurements were performed in a mixture of healthy subjects and patients with differing degrees of renal impairment with a wide range of GFRs. Serum was obtained from blood samples to enable a reference GFR to be calculated. Blood spots were collected on filter paper at the same intervals (120, 180, and 240 min), allowed to dry, and then sent through the post. Serum and blood spots were analyzed simultaneously for each individual by automated reverse-phase high-pressure liquid chromatography. Standard linear regression analyses confirmed a good agreement ($r^2 = 0.953$) between the iohexol serum GFR and iohexol blood spots GFR. Bland–Altman analysis confirmed that there was no concentration bias. Paired comparisons (Wilcoxon's paired signed rank test) showed no significant difference between the two measurements. Capillary sampling is simple, effective, and significantly reduces the time and costs of performing plasma clearance GFR measurements. This approach will make the GFR measurement more accessible for clinical practice and large-scale epidemiological studies may become feasible.

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Early diagnosis of declining renal function facilitates intervention to delay the onset of end-stage renal failure. To diagnose renal disease, early and accurately precise diagnostic tools are necessary. Glomerular filtration rate (GFR) is the major test of renal function. The most effective measurement of GFR is the measurement of inert substances exclusively cleared from the body by glomerular filtration. Inulin introduced in the 1930s[1] is still often used as a reference substance; however, this method is not widely available.[2] Measurements of clearance of radiolabeled markers such as ^{51}Cr-ethylenediamine tetraacetic acid are also widely used but require specialized facilities, and repeated use of radioactivity is undesirable and is not suitable for all patients.

In the 1980s, the development of rapid and reliable measurements of the clearance of the non-ionic radiological contrast agent iohexol was described. Subsequently, many studies have validated the use of iohexol clearance as a measure of GFR. Iohexol clearance was compared with Cr-ethylenediamine tetraacetic acid clearance[3] and inulin in adults[4] and children[5] and was found to be almost identical. Its low acute toxicity,[6] easy handling, and stability recommend iohexol clearance as a reference method. So much so that it has been suggested that it may be a 'new gold standard'.[4] GFR measurement using iohexol requires the subject to remain in the clinic for up to 5 h for subsequent blood sampling. This is time consuming and expensive and makes such a process unrealistic for screening or large-scale epidemiological studies. Therefore, in the present study, we propose an outpatient finger-prick method for the determination of iohexol GFR, which is suitable for the clinical practice and also for use in large-scale studies through collection of capillary samples on filter paper.

RESULTS

In the subset of patients ($n = 41$) where posted blood spots were compared with not posted blood spots, no significant difference was observed, 67.1 ± 4.9 vs 69.0 ± 4.9 (mean GFR (ml/min/1.73 m^2) $\pm$ s.e.m. for posted and not posted).

The analysis of blood spiked with 50 μg/ml iohexol gave a recovery of 50.4 ± 1.4 μg/ml in blood spots and 45.6 ± 1.8 μg/ml in whole blood. Comparison of recovery from whole blood was no different from recovery from plasma after

Table 1 | Intra- and inter-assay variability of plasma and plasma spot assay

Concentration iohexol (µg/ml)	20.2	40.4	80.8	Mean
Intra-assay cv (%)				
Plasma, n=10	3.4	0.3	4.2	2.7
Plasma spot, n=10	3.3	3.8	3.5	3.5
Inter-assay cv (%)				
Plasma, n=14	6.3	6.2	5.6	6.0
Plasma spot, n=10	8.1	6.2	4.8	6.4
Intra-assay cv (%)				
Blood spots, n=6	3.6	6.7	2.6	4.3
Inter-assay cv (%)				
Blood spots, n=10	5.1	6.1	4.8	5.3

cv, coefficient of variance.

Figure 1 | Accuracy of blood spot GFR relative to serum GFR. (**a**) Correlation between iohexol serum GFR and blood spots GFR (ml/min/1.73 m²) calculated according to single-compartment analyses and corrected by the Brochner-Mortensen equation. (**b**) Analysis by Bland–Altman plot demonstrates that the difference between the two measurements (y axis), finger prick and serum showed a bias of −1.17, which did not significantly change with changes in the degree of GFR (x axis).

hematocrit (ht) correction. Iohexol was stable for up to 4 h at 37°C.

Precision

Analysis of plasma, plasma spots, and blood spots showed an intra-assay mean coefficient of variance of 2.7, 3.5, and 4.3%, respectively. The mean coefficient of variance for inter-assay variability was 6.0% for plasma, 6.4% for plasma spots, and 5.3% for blood spots (Table 1).

Method comparison

Comparison of GFR measurements using serum and blood spots by standard linear regression analysis confirmed a good agreement between the two techniques ($r^2 = 0.953$), and Bland–Altman analyses showed a bias of −1.17 with upper and lower limits of agreements (bias ± 2 s.d.) of 13.78 (confidence interval 16.52, 11.04) and −16.12 (confidence interval −13.38, −18.86), respectively (Figure 1a and b).

DISCUSSION

Iohexol has been shown to be a safe and reliable reagent in the measurement of GFR. However, it has required subjects remaining in clinic for up to 5 h. This paper describes a highly valuable alternative in the outpatient finger-prick method for the determination of iohexol GFR.

Validation experiments performed confirmed the feasibility of this method; no differences were seen whether the samples were posted or not; and recovery experiments demonstrated a near 100% recovery of iohexol from blood spot samples; furthermore, no difference was observed between recovery from blood and ht-corrected plasma, indicating minimal association of iohexol with blood cells. Both intra- and inter-assay variability was acceptable. Comparison of the serum iohexol GFR with blood spots GFR concluded that there was no significant difference between the two and there was no concentration-dependent bias in the correlation.

Blood spot analysis will also help facilitate the use of iohexol GFR in small children, where heel prick can be used. The standard assessment of a percutaneous central venous cathet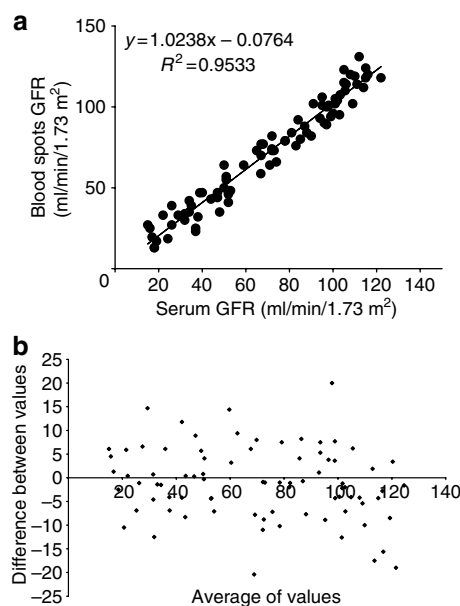er involves the injection of 1–2 ml of iohexol before taking a radiograph of a baby's chest, so that a GFR can be additionally performed. Blood collection from some renal patients is often more difficult owing to repeated venipuncture. The use of small volumes for blood spots may also be of use in these patients.

There are certain issues that need to be addressed before the system can be used as an 'outpatient' device, where the subject is responsible for taking the blood samples. First is the actual finger-prick method. In this study, blood was taken by a trained member of the staff; however, it is envisaged that the subjects should be able to do this and this is currently being trialed. Currently, spring-loaded lancets are used; however, a patent has been submitted for 'finger-grip' that holds the finger and lancet in place, allowing the whole procedure to be performed with greater ease. The timing of samples is an important issue in the measurement of GFR using iohexol. Blood was taken at 2, 3, and 4 h, and although the timing does not have to be accurate, the precise time does need to be recorded. Consequently, a small disposable timing device has also been developed, which was activated when iohexol was administered and again when the blood sample was taken, and was then returned to the laboratory with the sample. The 'finger-grip' and timer together would form a compact and inexpensive tool to allow this methodology to be used more widely.

There are conflicting data concerning how many time points are required for precise iohexol GFR measurements.[7–10] Consequently, in this study, comparison was made using three time points in both serum and blood spots.

Precision of less time points would require further study and comparison with formula-determined GFR.

In conclusion, the development and validation of a blood spot outpatient finger-prick method for the determination of iohexol GFR will improve GFR measurements in some patient groups and facilitate the epidemiological studies and screening projects, which will improve the detection of renal disease and our understanding of its distribution in the population.

MATERIALS AND METHODS

After obtaining informed consent, 22 healthy volunteers and 60 patient volunteers were included. The median age of all healthy volunteers (9 men and 13 women) was 41 years (range 21–62 years) and had levels of serum creatinine within the laboratory reference range. The patients (with differing degrees of renal impairment and a wide range of GFR-S) volunteering for the study (34 men and 26 women) were selected on the basis of serum creatinine with: 'normal' ($<130 \,\mu$mol/l; $n=56$ mean creatinine 96 μmol/l), medium (130–300 μmol/l; $n=21$ mean creatinine 170 μmol/l), and high ($>300 \,\mu$mol/l; $n=11$ mean creatinine 479 μmol/l) levels of serum creatinine. Creatinine was measured by a routine Bayer kit using the O'Leary modified kinetic Jaffe method on Bayer Advia 1650 equipment. Clearance was adjusted to the body surface calculated according to Du Bois and Du Bois[11] at 1.73 m^2.

Patients had a restricted diet (low protein intake and non-caffeinated drinks) for approximately 6 h before the test. Height and weight were recorded.

A zero-time blood sample was collected into BD Vacutainer SST II Advance tubes and an additional blood sample collected into ethylenediamine tetraacetic acid (for ht analyses) was collected before the intravenous injection of 5 ml of iohexol (Omnipaque 300, Nycomed Amersham Plc, Buckinghamshire, UK) and the exact time of the injection was recorded. The cannula was flushed with 10 ml of 0.9% saline. After the injection of contrast media, blood samples were collected into BD Vacutainer SST II Advance tubes at approximately 120, 180, and 240 min by separate venipuncture of the antecubital vein from the contralateral arm in which the iohexol was injected. The exact collection time was recorded. Blood spots were collected by finger-prick, taken by a member of staff, on the filter paper (Schleicher and Schuell Grade 903) at the same intervals as the blood samples and the exact collection time was recorded. Serum was separated from the blood samples and stored at -20°C.

Patients were supervised for 15 min for possible adverse reactions and then they were allowed to leave the department between sampling.

In a subset of 41 subjects, two sets of blood spots were analyzed. One set of spots was posted and one not posted. Both samples were analyzed at the same time. Ethical approval was given by Merton and Sutton Local Research Ethics Committee, number 71/00.

Iohexol standards and quality controls

Iohexol stock solution (647 mg/ml) was diluted into mobile phase (acetonitrile 3.5% pH adjusted to 2.5 with concentrated orthophosphoric acid) to give final concentrations of 0, 129, and 294 μg/ml iohexol and stored at 4°C.

Iohexol stock solution was diluted in serum to give quality controls with concentrations of 20.2, 40.4, and 80.8 μg/ml and stored at -70°C.

Samples analyses

Fifty-six microliters of serum samples, standards, and quality controls were treated with 850 μl of 5% perchloric acid to precipitate serum proteins. For blood spots, a 6.3 mm diameter punch of the blood spot (containing 11.2 μl of blood) was treated with 170 μl 5% perchloric acid. Samples were vortexed for 3 min, ultrasonicated for 15 min, incubated for 30 min on the bench at room temperature, and then spun at 14 000 g for 10 min. The volume of one injection for both serum and spots was 100 μl.

High-pressure liquid chromatography analyses

Iohexol was quantitated from serum and spots by automated reverse phase high-pressure liquid chromatography detector (Tosoh TSK 6040; pump-Laserchrom, Rochester, Kent UK; autosampler-Waters 717) using external calibration column—Nucleosil 120; C18; 5 μm; 20 × 4.6 mm—and guard column-Nucleosil 120 5 × 0.46 mm—Jones Chromatography (Hengoed, UK), maintained at 30°C, pump 1.5 ml/min, detector-wavelength 254 nm, and range 0.05. The second peak, which elutes at 7.5 min, was used for the calculation of the GFR (ml/min/1.73 m^2).

Method optimization

To determine recovery of iohexol and to investigate red blood cell uptake, blood from subjects was collected, spiked with iohexol (50 μg/ml), and recovery was compared between serum analyzed directly and blood spotted onto filter paper. Furthermore blood was spiked with 50 μg/ml iohexol and incubated for 0, 1, 2, 3, and 4 h at 37°C. Plasma was prepared from a separate aliquot of blood and recovery of iohexol was compared between blood and plasma, corrected for ht.

Precision. Plasma was spiked with 20.2, 40.4, and 80.8 μg/ml iohexol. Plasma was both analyzed directly and spotted on to paper. Analysis of intra- and inter-assay variation was investigated in both plasma and plasma spots.

Calculations of GFR

Blood spot iohexol concentrations were calculated assuming a fixed volume of blood on the filter paper. The equivalent value for serum is then calculated using the ht correction formula (iohexol)/(1−ht).

An analysis was performed as described previously.[3] In brief, single-compartment analyses equation 1 with Brochner-Mortensen correction was performed with three blood samples taken over a 4 h period.[12]

Clearance (Cl_1) according to one-compartment model was calculated from the least mean square semilog curve fit giving the elimination constant (k) and y-intercept (C_0) of the fitted line:

$$Cl_1 = \text{Dose}/(C_0/k)$$

The Brochner-Mortensen correction gives greater accuracy

$$Clp = 0.990778 \times Cl_1 - 0.001218 \times Cl_1^2$$

This final clearance was corrected for 1.73 m^2 standardized body surface area.

Statistical analyses. Standard linear regression was used to compare the two techniques. Results were analyzed by paired comparisons (Wilcoxon's paired signed rank test) and Bland–Altman analyses.

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