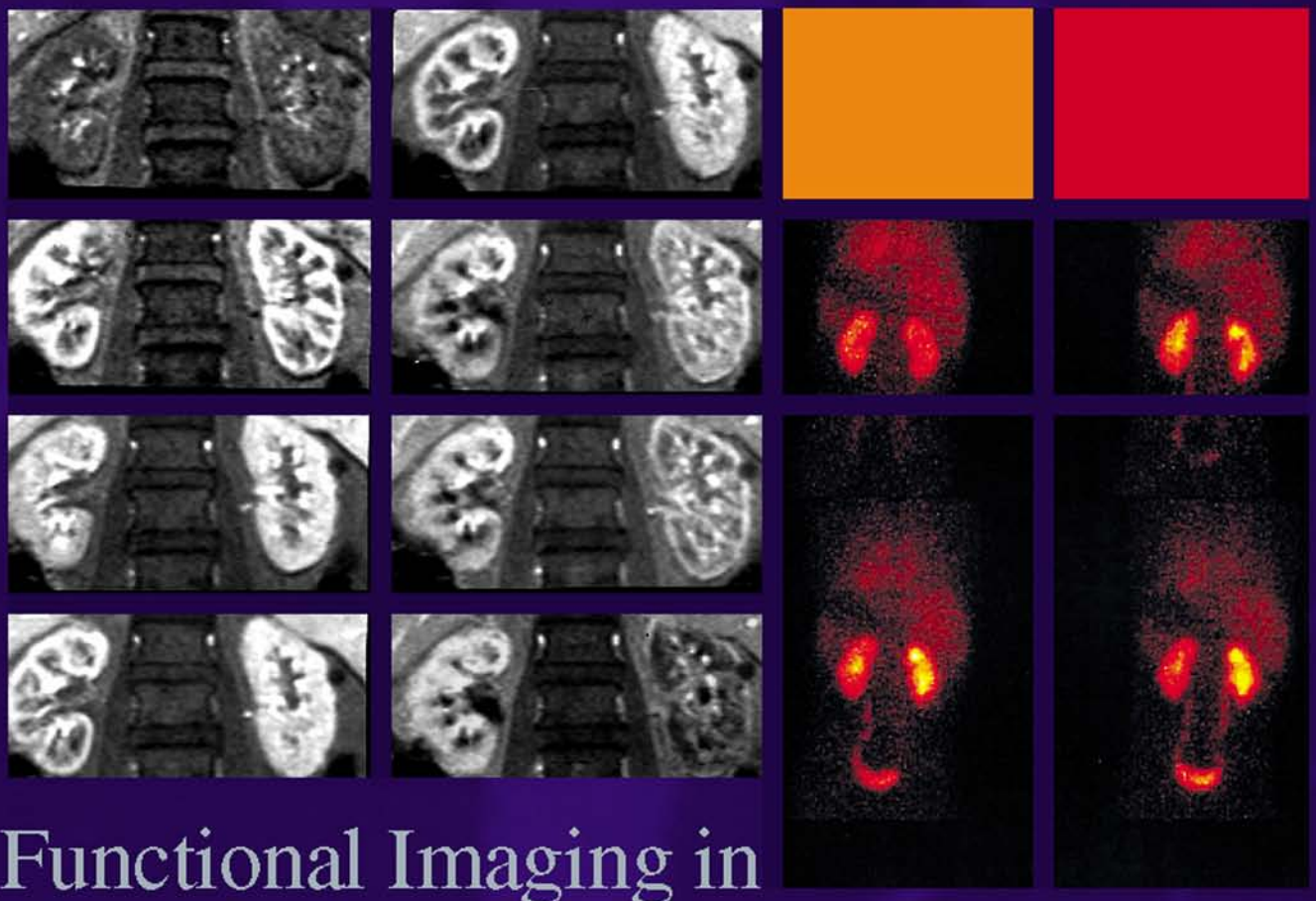

Functional Imaging in NEPHRO-UROLOGY

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Preface

In 1967, a first international symposium on radionuclides in nephro-urology was held in Liège (Belgium). The purpose of this symposium was to bring together a group of people with a common interest in the application of radionuclides in nephro-urology. Internists, radiologists, urologists, physiologists and others representing the basic and clinical sciences were brought together for intensive discussions. Since that time, similar meetings were organized by the International Scientific Committee of Radionuclides in Nephrourology (ISCORN) in New York (1971), Berlin (1974), Boston (1978), London (1981), Lausanne (1986), Williamsburg (1989), Chester (1992), Santa Fé (1995), Copenhagen (1998), Monterey (2001) and La Baule (2004). The final purpose of these meetings was the application of the radionuclide techniques in clinical fields such as hypertension, renal transplantation, hydronephrosis and infection. At the same time, a huge amount of methodological studies had given rise to new developments in nuclear medicine. It has been the role of ISCORN to chair consensus conferences, which resulted in a better standardization of radionuclide methods in fields such as measurement of renal clearance, evaluation of renal transit and drainage, application of captopril renography to renovascular disease, cortical scintigraphy in urinary tract infection in children and management of renal transplants.

The meeting, held in May 2004 in La Baule (France), was a kind of achievement. It was the feeling of the Committee that the time had come to bring together the different specialities involved in the strategy of uro-nephrological diseases and to evaluate the potential place of various techniques in the management of patients. The basic structure of the symposium was therefore centred on a series of clinical topics, all of them characterized by a significant number of controversial matters: determination of renal function in child and in adult, antenatally detected hydronephrosis, renal obstruction in adults, renovascular hypertension, renal infection in childhood. Radiologists, nuclear medicine physicians, physiologists, paediatric and adult nephrologists, paediatric and adult urologists, all eminent experts in their respective fields, developed the state of the art and constituted then a large panel for long and well-structured discussions with the audience. The most up-to-date developments of the traditional methods were presented by the different speakers, while new techniques, such as functional and molecular imaging with MR, CT and PET appeared as promising approaches. What came out of these multidisciplinary sessions is remarkably similar for all topics, namely a critical appraisal of the traditional strategies of management and a series of potential new directions which might, in the near future, significantly change the clinical management of the patient.

It appeared therefore that the moment was well chosen to reassemble this huge amount of information within a book under the general title of 'The Role of Functional Imaging in Nephro-urology'. The chapters correspond to the five clinical sessions and for each topic, the contributors provided a detailed and referenced overview of their expertise, completed by a rich iconography.

This book, by its multidisciplinary approach, is a 'première' and will provide outstanding information to radiologists working on child and adult, to nuclear medicine physicians, to internists and paediatricians, to nephrologists and urologists specialized in child and adult.

We want to express our sincere thanks for help to the other members of the ISCORN committee: Donald Blaufox (New York, USA), Keith Britton (London, UK), Eva Dubovsky (Birmingham, USA), Belkis Erbas (Ankara, Turkey), Jörgen Frökiaer (Aarhus, Denmark), Joseph V. Nally (Cleveland, USA), Patrick O'Reilly (Stockport, UK), Pilar Orellana (Santiago, Chile), Monica Rossleigh (Sydney, Australia), Michael Rutland (Auckland, New Zealand) and Andrew Taylor (Atlanta, USA). Thanks also to the experts of all specialities who contributed by their outstanding presentations to the success of this multidisciplinary event. Their lectures were the starting materials for the different chapters of this book. All of this would not have been possible without the help of the organizing committee (Joseph Lecloirec, MD and Mrs Maïté Lepelletier) who did a great job in making this conference one of the most exciting meetings ISCORN has ever experienced. Finally sincere thanks to Tyco France and Tyco USA whose role in sponsoring the meeting has been essential.

Alain Prigent, Paris, France
Amy Piepsz, Brussels, Belgium
Editors

Measurement of renal function in health and disease

1 Introduction

Alain Prigent

Operational definition of renal function

The level of the glomerular filtration rate (GFR) is generally accepted as the best overall index for the complex functions of the kidney in health and disease.[1] This agreement holds on to functional, pathological, clinical and prognostic arguments. The functional coupling between GFR and tubular function especially relies upon the 'positive' glomerulotubular balance and the 'negative' tubuloglomerular feed-back, which ensure an integrative regulation of the whole nephron function. Similarly, the GFR decrease correlates with the extent of tubulointerstitial fibrosis and/or tubular atrophy in chronic renal diseases.[2] GFR being reduced prior to the onset of symptoms of renal failure, its assessment enables earlier diagnosis and therapeutic interventions in patients at risk. Thus the level of GFR is a strong predictor of the time of onset of kidney failure as well as the risk of complications of chronic kidney disease.[1] Many techniques, using either chemical or radiopharmaceuticals, exist providing either estimates or true measurements of the global GFR.

In case of asymmetrical renal disease the determination of the individual renal function requires a global GFR measurement to be combined with the assessment of the split renal function (e.g., expressed in percentages of the global function) by a noninvasive imaging modality. Although renal scintigraphy is presently the most often used because of its widespread availability, low cost (compared to the alternative modalities of computed tomography and magnetic resonance imaging), and absence of side effects from the tracers,[3] new applications of computed tomography (e.g., multidetector CT, electron beam computerized tomography) and magnetic resonance imaging appear promising (see Chapters 15 and 17).

The clearances of some tubularly secreted organic anions, such as *p*-aminohippuric acid (PAH), ^{131}I- or ^{125}I-ortho-iodohippurate (OIH), or even ^{99m}Tc-mercaptoacetyltriglycine (MAG 3), are referred to as the effective renal plasma flow (ERPF). GFR is related to ERPF by the expression:

$$GFR = ERPF.FF/EF_{oa}$$

where EF_{oa} is the extraction fraction of the used organic anion (EF_{oa} = ERPF/RPF, RPF being renal plasma flow), and FF the filtration fraction (FF = GFR/RPF, about 0.20 in normal humans). However, as FF changes occur in certain clinical circumstances (e.g., proteinuric glomerulopathy, ischaemia, postischaemic injury after transplantation, renovascular hypertension, acute urinary obstruction, …), RPF changes do not always parallel GFR changes. Moreover, EF_{oa} varies dramatically and unpredictably in numerous conditions, especially in chronic renal diseases. As an example, the extraction fraction of PAH, considered as the gold standard molecule for ERPF measurement, is 0.92 ± 0.03 (mean $\pm$ SE) in normal volunteers[4] but may decrease to an average of 0.80 in benign essential hypertension,[5] 0.75 in patients treated with cyclosporin,[4] 0.70 in proteinuric glomerulopathies,[6] or to 0.20 in ischaemic acute renal failure and to 0.10 in the recovery period.[7] Moreover, in all these cases the standard deviation of the mean EF_{oa} is about 0.10 to 0.15, indicating a wide range of variation between individual data. In renovascular disease, where the renal function is asymmetrical, EF_{oa} of PAH is about 0.55 and 0.75 in the stenotic and contralateral kidney, respectively, and decreases further to 0.35 and 0.65, respectively, after administration of captopril.[8] Even with the most sophisticated curve-fitting procedures most methods are too imprecise for accurate prediction of EF_{oa} in a given individual.[4]

Renal function in health

Notwithstanding the great variability of GFR even in healthy individuals due to many physiological factors (e.g., body size, gender, age, salt and dietary protein intakes, diurnal variations), normal ranges of GFR have been reported. This variability can be reduced by taking into account body surface area ('normalization' to 1.73 m^2). When using the 'classical' gold standard of inulin clearance,[9] the mean values of GFR in young adults are 127 ml/min/1.73 m^2 in men and 118 ml/min/1.73 m^2 in females with a standard deviation of approximately 20 ml/min/1.73 m^2, while when using ^{51}Cr EDTA (ethylenediaminetetraacetic acid) plasma clearance,[10] the normal mean ($\pm$SD) GFR is 105 ($\pm$25) ml/min/1.73 m^2 (no gender difference after correction for BSA). With regards to transversal studies,[10–13] GFR linearly decreases by approximately 1.0 ml/min/1.73 m^2 per year with large interindividual variation even among 'healthy' individuals. Indeed, regarding longitudinal studies,[14] one-third of the healthy elderly subjects

has no absolute change, another third has a progressive but small decline, and in the last third of elderly GFR declines to 50–70% of the maximum GFR value. In one-month aged neonates,[15,16] the mean GFR is about half the adult value (55 ml/min/1.73 m^2) and increases progressively until 18 months–2 years. Between 2 and 17 years of age, expressed as ml/min/1.73 m^2, the GFR remains constant, with a mean value of 114 ml/min/1.73 m^2 (SD: 24 ml/min/ 1.73 m^2), similar to the value in adults.

In normal individuals, the reactive increase in GFR (120–140% of the baseline value) within the 2 hours following an oral protein load (e.g., 300–500 g of cooked beef) is defined as 'functional renal reserve'.[17] Subsequently, similar increases in GFR were reported[18] with either gluconeogenic amino acids (50–75 g within 3 hours) or dopamine (1.5–2 μg/kg/min for 2 hours) infusion. Although it was initially thought that this 'reserve' was lost in the presence of early renal impairment (i.e., not diagnosed by plasma creatinine test), these findings were not confirmed in many later series. Expressed as a percentage of baseline GFR value, the 'functional reserve' does not decrease in kidney diseases (see reference 19). Apart from this diurnal variation due to meal intake, there is a circadian rhythm of GFR[20] with a maximum around 1 pm, a minimum around 1 am, and a relative amplitude ([max – min]/mean) of about 30% for inulin clearance and 20% for creatinine clearance. The nutritional status also affects GFR,[21] especially dietary intakes of proteins, calories (whatever the nutriments), and sodium (an important determinant of extracellular fluid volume, ECFV). For an example, GFR increases to about 140% of its baseline value during pregnancy in relation to an increase in ECFV.

At the borderline between health and disease, the compensatory hyperfunction of the remnant kidney in donors restricts, partly the functional lost. Thus GFR (^{125}I-iothalamate urinary clearance measurements) is about 60% (69 ± 4 ml/min/1.73 m^2) and 70% (78 ± 5 ml/min/ 1.73 m^2) of the predonation value (111 ± 6 ml/min/ 1.73 m^2) at about one month and 5 years after the nephrectomy, respectively.[18]

Renal function in disease

The aim of the following chapter is to go through every issue related to the measurement of renal function and to define which methods are adequate for which patients'. However, we agree that no single test of GFR is perfectly suited for every clinical and research application. Thus, the goal should be to propose a specific clinical question (screening, confirming, following, …) the most accurate, precise, safe, convenient and cost-effective (not only the cheapest) method.

Recently, the Kidney Disease Outcome Quality Initiative (K/DOQI) of the National Kidney Foundation (USA) has proposed guidelines,[1] among which one is dedicated to the definition and classification of stages of chronic kidney disease (guideline 1) and another to its evaluation by estimation of GFR (guideline 4).

GFR plays a cornerstone role in the definition of chronic kidney disease (CKD), since CKD is defined on two criteria, one of which being a decreased GFR:

1. **Kidney damage for 3 months at least**, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifest by either pathological abnormalities, or markers of kidney damage (including abnormalities in the composition of the blood or urine, or abnormal imaging tests);
2. **GFR lower than 60 ml/min/1.73 m^2 for 3 months at least**, with or without kidney damage (as defined in criteria 1).

Similarly, the GFR level is used for the stage definition of CKD:

- Stage 1: with normal or increased GFR: GFR ≥ 90 ml/min/1.73 m^2
- Stage 2: GFR between 60 and 89 ml/min/1.73 m^2 (mild)
- Stage 3: GFR between 30 and 59 ml/min/1.73 m^2 (moderate)
- Stage 4: GFR between 15 and 29 ml/min/1.73 m^2 (severe)
- Stage 5: GFR < 15 ml/min/1.73 m^2 (renal failure).

For the 'estimation' (not the measurement) of GFR, the recommendation is to use prediction equations taking into account serum creatinine concentration and some of the variables, which determine the creatinine production, such as age, gender, body size, ethnicity, etc. Estimating GFR by prediction equation based on serum creatinine is more reliable (i.e., more accurate and more precise) than measuring 24-hour creatinine clearance, mainly because of interpatient and intrapatient variability in creatinine tubular secretion and inability of most patients to accurately collect timed urine samples.[22–24] The day-to-day coefficient of variation of creatinine clearance has been reported as high as 27% in a routine clinical setting.[25]

The two recommended formulae for predicting either creatinine clearance or GFR are, for adult patients, the Cockroft–Gault equation[26] and the 'abbreviated' MDRD study equation (MDRD for Modification of Diet in Renal Disease), respectively and in children, the Schwartz[27] and Counahan–Baratt[28] equations, respectively (Table 1.1). However, these recommendations do not answer the question 'which methods for which patients' since the guideline about *estimation* of GFR only states that 'all four formulae reviewed provide a marked improvement over serum creatinine alone' for clinical assessment of kidney disease. Moreover, K/DOQI guidelines acknowledge, firstly that 'estimation of GFR and creatinine clearance from serum creatinine is critically dependent on calibration of the serum

Table 1.1 Equations recommended by the National Kidney Foundation (NKF/DOQI) to predict creatinine clearance and GFR based on serum creatinine

Adult	
Cockcroft–Gault[26]	C_{CR} (ml/min) $= \frac{(140 - \text{age}) \times \text{weight}}{72 \times S_{CR}} \times (0.85 \text{ if female})$
Abbreviated MDRD[78]	GFR (ml/min/1.73 m^2) $= 186 \times (S_{CR})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African-American})$ $= \exp(5.228 - 1.154 \times L_n [S_{CR}] - 0.203 \times L_n (\text{age}) - (0.299 \text{ if female}) + (0.192 \text{ if African-American}))$
Children	
Schwartz[27]	C_{CR} (ml/min) $= \frac{0.55 \times \text{length}}{S_{CR}}$
Counahan–Baratt[28]	GFR (ml/min/1.73 m^2) $= \frac{0.43 \times \text{length}}{S_{CR}}$

S_{CR}, serum creatinine in mg/dl (to convert mg/dl to μmol/l multiply by 88); C_{CR}, creatinine clearance; weight in kg; length in cm; age in years.

creatinine assay', and secondly that, 'in certain clinical situations, *clearance measures* may be necessary to estimate GFR'.

Numerous methods are used to measure creatinine, mainly colorimetric (based on Jaffe' reaction) or enzymatic assays. The more commonly used colorimetric methods systematically overestimate creatinine concentrations by about 20% compared to enzymatic measures (lower interference with noncreatine chromogens) and by 20% to 80%, when compared to high-performance liquid chromatography (HPLC) and dilution mass spectrometry measures, which should approximate 'true creatinine'.[1] The College of American Pathologists[29] reported that in laboratories surveyed in 1994, creatinine was overestimated on average by 13% to 17% (0.12–0.17 mg/dl or 11–15 μmol/l). Serum creatinine assays on the same samples were 0.23 mg/dlL (20.3 μmol/l) higher at the White Sands Laboratory (Third National Health and Nutrition Examination Survey, NHANES III) than at the Cleveland Clinic (MDRD study), although both laboratories used Jaffe' reaction-based methods but on different auto-analysers.[30]

Without any correction of this bias by a calibration factor (0.81), the prevalence of low GFR (30–60 ml/min/1.73 m^2) in NHANES III would have been erroneously increased fourfold (12.5 versus 3.2%).[31] Recently, the French Society of Clinical Biology assessed interassay variation and accuracy of blood creatinine measurements as well as the effect of the standardization of calibration procedures on interassay variation. Thirty frozen human sera and three certified reference materials were analysed by 17 creatinine assays (12 colorimetric, four enzymatic and one HPLC).[32] Most of the commercially available methods had inaccuracy higher than 10% for serum creatinine lower than 1.7 mg/dl (150 μmol/l). The median dispersion factor was 14% between 0.5 and 1.7 mg/dl (45–150 μmol/l, the range of mild to moderate renal impairment) and 8% between 2.9 and 4.0 mg/dl (250–350 μmol/l). Moreover, the bias was not constant over the clinical range of serum creatinine, enzymatic assays producing lower results than colorimetric ones for low creatinine levels, but conversely higher results for high creatinine levels. Due to the lack of a standardized calibration procedure using several concentrations (with at least one between 0.1 and 1.7 mg/dl or 90–150 μmol/l), the intra-assay variation is too high to allow prediction of creatinine clearance or GFR from serum creatinine levels, contrarily to K/DOQI guidelines recommendations. Similar conclusions have been reached by other groups working on the prevalence of low GFR in nondiabetic Americans[31,33] or on the risk factors on renal function (Prevention of Renal and Vascular End-stage Diseases study, PREVEND).[34] Although the K/DOQI working group has chosen an estimated GFR cutoff of less than 60 ml/min/1.73 m^2 for diagnosing chronic kidney disease in the absence of kidney damage, an improvement in estimating GFR from MDRD formula could be to include creatinine assay methods as a covariable in the prediction equation used[30,32] keeping in mind the interlaboratory variation in measurement of serum-creatine.

Since creatinine clearance overestimates GFR, the estimates given by the Cockcroft–Gault and Schwartz formulae are biased too. Thus, in a large sample of more than 500 adults with a wide range of GFR (up to approximately 90 ml/min/1.73 m^2), Cockcroft–Gault formula overestimates GFR, directly measured by ^{125}I-iothalamate urinary clearance, by 23%.[35] With Schwartz formula, the bias increases markedly in children with low GFR, with overestimation up to 32% and 67% for GFR (^{125}I-iothalamate clearance) between 31–50 ml/min/1.73 m^2 and lower than 30 ml/min/1.73 m^2, respectively.[36]

Another issue is the reliability of the claimed statement that the four formulas recommended provide a clinically useful estimate of GFR' in the K/DOQI guidelines. This statement relies on a rather optimistic definition of what is an accuracy sufficient enough for good clinical decision-making. Thus, the accuracy was defined as the percent of GFR estimates within 30% of measured GFR (i.e.; in the 70–130% range of GFR measured by radionuclide tracer, inulin or iohexol clearances). Results of about ten studies (see reference 1) assess-

ing accuracy in adults and children indicated that a quarter of the patients have estimated GFR by Cockcroft–Gault and Schwartz formulae, respectively, out of this large range of uncertainty (about 60% of the measured, 'true' GFR). Although the claimed clinical usefulness of Cockcroft–Gault and Schwartz formulae are questionable regarding such a low accuracy, abbreviated MDRD and Counahan–Baratt formulae are more efficient with only 10% and 15–30% of estimated GFR, respectively, which did not fall into the 30% accuracy range.

Assuming that a GFR prediction formula derived from a patient population will be valid when applied to another population may be erroneous. For example, Cockcroft–Gault formula systematically overestimates GFR in obese[37] or oedematous individuals[38] and is inaccurate in diabetic patients.[39] Similarly, Schwartz formula is not reliable in children with insulin-dependent diabetics mellitus,[40] with liver disease,[41,42] and after liver transplantation.[43] Even the more recent abbreviated MDRD formula was recently reported as inaccurate for GFR estimation in healthy potential kidney donors,[11,44] a conclusion not so surprising since the patients included to derive the MDRD formulae had GFR up to 90 ml/min/1.73 m^2 only. The same conclusion is predictable for the early stage of CKD in diabetic patients, where GFR may be normal or even increased.[45] Therefore, measurements of GFR are needed to identify early decline or increase in kidney function, especially in patients at high risk for renal functional impairment (e.g., diabetes, renal transplant rejection, systemic lupus erythematosus, etc.).[1,46,47]

In clinical situations in which the average rate of production of creatinine is unpredictable from the variables used in the prediction formulae, GFR measurements by clearance methods are mandatory. Estimates will be unreliable in severe malnutrition, obesity, prolonged parenteral nutrition, corticotherapy (e.g., chronic kidney and liver disease and transplantation), neuromuscular diseases, paraplegia or quadriplegia and vegetarian diet (low creatinine dietary intake). More problematic, the fundamental assumption of the MDRD formula that age, gender, ethnicity and blood urea nitrogen (BUN) account for creatinine production, is invalid in patients with advanced renal failure, and the use of MDRD formula in these patients might introduce biases.[48]

Even if the prediction equations to estimate GFR had been validated by tests in adults (or children), elderly, diabetics and nondiabetics, high-risk patients (e.g., for CKD or cardiovascular disease), transplant recipients and among different ethnicities (and not only African/Mexican/Caucasian/American), their clinical use would be limited. The most important hurdle remains that they were derived from adjustment variables (e.g. age, gender, height and body weight) more effective for detecting interpatient differences than intrapatient time changes. Consequently, and as specified in the K/DOQI guidelines, 'estimates of GFR based on serum creatinine will only enable the detection of substantial progression (>25% to 50% decline) … and will lead to false measures of lower degrees of progression'. In this context, it should be recalled that the coefficient of variation (CV) of inulin clearance measured on different days in the same individual (with invasive bladder catheterization, no data using spontaneous voiding) is approximatively 7.5%,[49] the median intertest (3 months interval) CV of ^{125}I-iothalamate urinary clearance (spontaneous voiding) is 6.3%,[50] and the total day-to-day CV of ^{51}Cr-EDTA plasma clearance (no urinary collection) is 4.1% and 11.5% in patients with a GFR > or ≤ 30 ml/min, respectively.[25]

In 1989, Andrew S. Levey, the first author of many papers published by the MDRD study working group, had already concluded in a review about the use of GFR measurements to assess the progression of renal disease[49] that 'estimation of GFR from renal clearance of radioisotope-labeled filtration markers, using a bolus infusion and spontaneous bladder emptying, is accurate, precise, and more convenient than the classical inulin clearance techniques, and that measurements of GFR should be included in clinical research'.

The next chapters will analyse the chemical and radionuclide techniques available to measure GFR in adults and children and propose answers or suggestions for the selection of the most appropriate methods in different clinical settings both in adults and children.

Besides prediction formulae based on serum creatinine, Joe Nally will discuss serum cystatin C, which has been suggested for detecting early changes in GFR especially in children, liver disease and kidney transplant, where creatinine-based formulae are inaccurate. However, sample sizes are limited and results are still conflicting.[51–55] The use of iodine contrast media (e.g., iohexol) as a nonradioactive substitute in urinary and plasma clearance methods[56–62] has been proposed to measure GFR. However, expensive and time-consuming HPLC is required to allow the use of a small injection dose (unlikely to induce adverse effects except allergic reactions) and accurate measurement of low serum concentrations. X-ray fluorescence method is less sensitive and accurate, and needs a higher sampled blood volume.

Table 1.2 Which methods for which patients

Applications	*Clinical settings*
Screening	Prevalence of low GFR • general population • high-risk patients (diabetes, CVD, etc.)
Confirming	Inaccurate/doubtful estimated GFR (e.g., chronic rejection), prognostic information (e.g., SLE), need for therapy or additional diagnostic test
Following	Disease progression, therapeutic follow-up, need for dialysis or transplantation (very low GFR)
Investigating	Renal toxicity, renal clearance of drugs to guide dosing, renal functional reserve, normal values (e.g., ageing)

The point of view of the nephrologist and the paediatric nephrologist will be developed by M. Laville and J.P. Guignard, respectively.

Which methods should be used for well-defined clinical conditions? The methods may differ strongly, depending on the type of clinical application concerned. Table 10.2 lists the clinical settings and the corresponding field of application. Answering such questions needs to consider the criteria for the choice, which may have different weights in the decision-making process, depending on the main aim of the test (e.g., screening patients at risk or confirming the need for dialysis or transplantation).

In general, the tests that are most accurate (low bias compared to the standard) and precise (good reproducibility and small difference for a significant change) are also those that are less convenient (simplicity, safety, availability, cost).

E. Durand will present radionuclide clearance methods, either urinary (i.e., either constant infusion or intravenous/subcutaneous single injection) or plasma clearance (i.e., either infusion-equilibrium or single injection) and some external detection based methods, used to measure split renal function, such as the fraction injected dose[63–69] uptake and the functional uptake rate.[70]

A. Piepsz will consider issues more specific to children, especially the advantages and limitations of different plasma clearance techniques (i.e., slope/intercept method, 'only-slope' method,[71,72] one-sample versus two–three sample method, and infusion-equilibrium method[73,74]), the question of normalization/indexation to body surface area (BSA) or extracellular fluid volume (ECFV),[75,76] and the question of the 'switch' from children- to adult-estimated GFR equations in adolescents and young adults.[77]

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2 Assessment of GFR: chemical techniques and prediction equations

Joseph V Nally

Introduction

The kidney plays a vital role in maintaining total body homeostasis by having both excretory and endocrine functions. The excretory functions are more readily recognized as the kidney rids the body of potential uremic toxins and maintains vascular volume, critical fluid–electrolyte and acid–base balance. The kidney also plays an endocrine role related to the production of such important hormones as renin, 1,25 vitamin D, erythropoietin, etc. Overall, the level of the glomerular filtration rate (GFR) is generally accepted as the best index for the complex functions of the kidney in health and disease.

The Kidney Disease Outcome Quality Initiative (K/DOQI) of the National Kidney Foundation was established to define and classify chronic kidney disease (CKD) to assist the clinician in earlier recognition and treatment of CKD and its complications. These K/DOQI clinical practice guidelines identified GFR as the keystone for the definition and staging of CKD. Table 2.1 lists the two general criteria for defining CKD.[1] In brief, CKD is defined as kidney damage manifest by abnormal renal pathology, urinalysis (albuminuria/haematuria), renal imaging studies or abnormal blood work. In addition, CKD may be defined as a reduction of GFR less than 60 ml/min for at least 3 months – with or without obvious kidney damage. Table 2.2 lists the stages of CKD based upon level of GFR.[1]

Table 2.1 Definition of chronic kidney disease (CKD)

Structural or functional abnormalities of the kidneys for ≥ 3 months, as manifested by either:

1. Kidney damage, with or without decreased GFR, as defined by:
   a. pathologic abnormalities
   b. markers of kidney damage, including abnormalities in the composition of the blood or urine or abnormalities in imaging tests
2. GFR <60 ml/min/1.73 m^2, with or without kidney damage

Table 2.2 Staging of CKD

Stage	*Description*	*GFR (ml/min/1.73 m^2)*
1	Kidney damage with normal or increased GFR	>90
2	Mild decrease in GFR	60–89*
3	Moderate decrease in GFR	30–59
4	Severe decrease in GFR	15–29
5	Kidney failure	<15 or dialysis

*May be normal for age.

The K/DOQI report recommends that serum creatinine (SCr) and an estimated GFR (eGFR) derived from prediction equations be reported to the clinician. Hence, a detailed understanding of the methodologies to measure or estimate GFR is vital for today's clinician as GFR is the benchmark for defining and staging CKD.

In order to measure GFR, one must measure the clearance of an agent that is excreted via the kidney by GFR alone. It is important to recognize the characteristics of an ideal agent for measuring GFR. The agent should be safe, nontoxic, and freely filterable at the glomerulus without appreciable tubular reabsorption or secretion. The latter quality separates these GFR agents from effective renal plasma flow (ERPF) agents which are cleared via the kidney by both GFR and active tubular secretion. This chapter will focus on those methodologies that use chemical techniques for assaying those substances in blood and urine which measure or estimate GFR. The reader is referred to Chapter 3 which describes the radionuclide assessments of GFR.

Chemical techniques

Inulin

Inulin has long been regarded as the 'gold standard' of exogenously administered markers of GFR. However, its scarcity and high cost have greatly diminished its usefulness and it is now generally of historical interest only.

Inulin is a fructose polysaccharide (molecular weight 2200 Daltons) found in such tubers as the dahlia, the Jerusalem artichoke and chicory. It possesses the ideal characteristics of a GFR agent as it is inert, freely filtered at the glomerulus and neither reabsorbed nor secreted by the renal tubules. Inulin may be measured in plasma and urine by one of several colorometric assays.

Utilizing inulin to measure GFR was originally developed and championed in the 1930s by Homer Smith, the father of renal physiology.[2] The technique has been used by many investigators over the ensuing decades and has had little modification. The procedure uses a bolus and infusion technique in a water-loaded patient. Urinary clearance was calculated using three to five periods. The coefficient of variation between the clearance periods was 10% and coefficient of variation of inulin clearance measured on different days in the same patient approximate 7.5%.[3]

The urinary clearance techniques were both cumbersome and inconvenient. To avoid problems with collection and/or bladder catheter placement, many investigators turned to plasma disappearance technique using either constant infusion or bolus injection.[4] Nevertheless, the decline in the use of inulin as a GFR marker has been largely attributable to its scarcity, cost and cumbersome methodologies.

Iohexol

Given the difficulty and expense of measuring GFR using inulin infusion clearance techniques, radiological contrast media such as iothalamate diatrizoate and iohexol have been suggested as alternatives which can be measured using chemical techniques. These substances may serve well as GFR markers as they fulfil the ideal characteristics of such agents.

Iohexol has been introduced as nonionic low-osmolar radiologic contrast medium that can be analysed in serum by using HPLC and X-ray fluorescence (Renalyzer) techniques. Over the past two decades, these measurements have opened up the field for use of low-dose iohexol (i.e., 10 ml versus the traditional 100 ml for X-ray purposes) as a GFR marker.[5] Slow intravenous injection with a small dose of iohexol for a clearance procedure is not nephrotoxic as is the case of high-pressure injection of larger amounts of contrast. In a large study of nearly 4000 iohexol clearance measurements, few adverse reactions were noted.[6] Several investigators have found a good correlation with plasma clearance of iohexol with that of inulin, chromium EDTA and technetium DTPA.[7] Furthermore, Brown and O'Reilly made a detailed study utilizing bladder catheterization and the classical continuous infusion techniques and demonstrated an excellent correlation between the renal clearances of iohexol and inulin.[8] These techniques utilize the plasma disappearance methodologies of Brochner–Mortensen, using three to four plasma samples after injection.[9] Other investigators have also demonstrated acceptable estimates of GFR using the single plasma sample model of Jacobson.[10]

In a cohort of patients with a wide range of kidney function (GFR 14–104 ml/min), Gaspari and colleagues demonstrated a high level of precision of the iohexol plasma disappearance technique using multiple plasma samples measured by HPLC.[11] Overall, the mean intra-individual coefficient of variation and reproducibility was 5.7 and 6.3% – even in patients with GFRs <40 ml/min. Swedish investigators studied iohexol plasma disappearance measured by X-ray fluorescent techniques using the single sample model of Jacobson in patients with renal disease. They concluded that single samples at 4 h for GFR >50 ml/min, 7 h for GFR 20–50 ml/min, and 24 h for GFR <20 ml/min gave values in good agreement with those based upon a four-sample slope clearance of iohexol.[10] In contrast to these patients with impaired kidney function, Australian investigators studied patients with diabetic nephropathy and preserved GFR and suggested that the Brochner–Mortensen modified one compartment model was preferred in patients with GFR >60 ml/min.[12]

In patients with GFR >40 ml/min, Cr^{51}-EDTA was compared to iohexol clearances using two different methods of iohexol analysis, HPLC and X-ray fluorescence, referring both to multisample and single-sample calculations.[7] The single- and multiple-point clearances determined by HPLC and X-ray fluorescence compared to Cr^{51}-EDTA correlated highly (R >0.92 in all). The authors concluded that iohexol and Cr^{51}-EDTA were comparable as GFR markers for multiple point clearance measurements. The single-sample method of GFR for patients with GFR >40 ml/min can be used with high accuracy. The precision and accuracy of X-ray fluorescence analysis of low concentrations of iohexol were less than the more costly HPLC analysis.

In aggregate, many clinical research centres throughout Europe favour the measurement of GFR using either the Cr^{51}-EDTA or iohexol plasma disappearance clearance techniques. Cost may be a factor in the measurement of the latter if the more precise and costly technique using HPLC is used.

Creatinine

SCr is the most widely used assay to measure the presence and progression of CKD.[13] The predictive equations for GFR are also critically dependent on the accuracy and reproducibility of the measurement of SCr.[14] Creatinine is derived from the metabolism of creatine in skeletal muscle. Creatinine is released into the circulation at a relatively constant rate and has a stable plasma concentration in the steady state. Endogenous creatinine production may vary with muscle mass, age, gender, ethnicity and nutritional status of the subject. As noted, creatinine is freely filtered by the glomeruli and it is neither reabsorbed nor metabolized by the renal tubular cells. However, 10–20% of urinary creatinine

may be derived from tubular secretion from the proximal tubules by organic cation secretory mechanisms.

The most widely used assay to measure SCr is based on the modified kinetic Jaffe reaction. The Picric-acid–Jaffe reaction has been recognized as overestimating SCr in normal individuals by 20–30% relative to HPLC or mass spectroscopy measurements because of 'noncreatinine chromogens'.[15,16] In contrast, there is a negligible amount of 'noncreatinine chromogens' in urine – which might lead to an underestimate of the creatinine clearance. By coincidence, the overestimation of SCr due to 'noncreatinine chromogens' provides a nearly equal balance for the tubular secretion of creatinine such that the measured creatinine clearance is a good estimate of GFR in healthy subjects. In patients with progressive CKD, the tubular secretion of creatinine is more robust such that the creatinine clearance will overestimate GFR. SCr may be increased in selective circumstances which would not reflect a true reduction in GFR. Certain drugs (e.g., trimethoprim, cimetidine) may increase SCr by decreasing the tubular secretion of creatinine. Other substances or drugs may interfere with the alkaline-picric colorimetric assay as they are recognized as creatinine chromogens (e.g., acetoacetate in diabetic keto-acidosis, cefoxitin, flucytosine, etc.). Endogenous creatinine production may be increased in circumstances such as rhabdomyolysis or catabolic states which might increase SCr.

Precise measurement of SCr is critical in measuring or estimating GFR. Advances in clinical chemistry have led to the development of the modified kinetic rate Jaffe reaction and enzymatic methods which can be calibrated to avoid measurement of 'noncreatinine chromogens.' In 1994, the College of the American Pathology (CAP) surveyed 700 laboratories and noted that the differences in calibration of SCr assays accounted for 85% of the difference between the SCr measurements.[17] The lab surveys overestimated SCr by 13–17% with considerable interlab variation.

In 2003, a similar survey by CAP of 5624 labs noted significant bias variability related to instrument manufacturer, rather than the type of alkaline picric acid or enzymatic methodologies.[18] In the USA, the National Institutes of Health (NIH)/National Kidney Disease Education Program (NKDEP) recommends that laboratories calibrate their SCr measurements to a Cleveland Clinic Lab standard as it has been the core Renal Function Laboratory in the development of the Modification of Diet in Renal Disease (MDRD) prediction equations for estimating GFR.[18] The precise measurement of SCr is crucial in estimating GFR using predictive equations as a small error in SCr may translate into a more substantial variability in GFR estimates.

Even if creatinine is measured accurately, both SCr and creatinine clearance have significant limitations in estimating true GFR. Equations based on SCr, age, gender and other variables perform much better at predicting GFR than SCr alone. Indeed, from the pioneering days of Homer Smith, the nonlinear (i.e., curvilinear) relationship between increasing SCr and falling GFR was recognized. Creatinine clearance was recognized as a better index of GFR because it takes into account the urinary creatinine which approximates the endogenous production of creatinine based upon muscle mass, age, gender and ethnicity. However, there are two

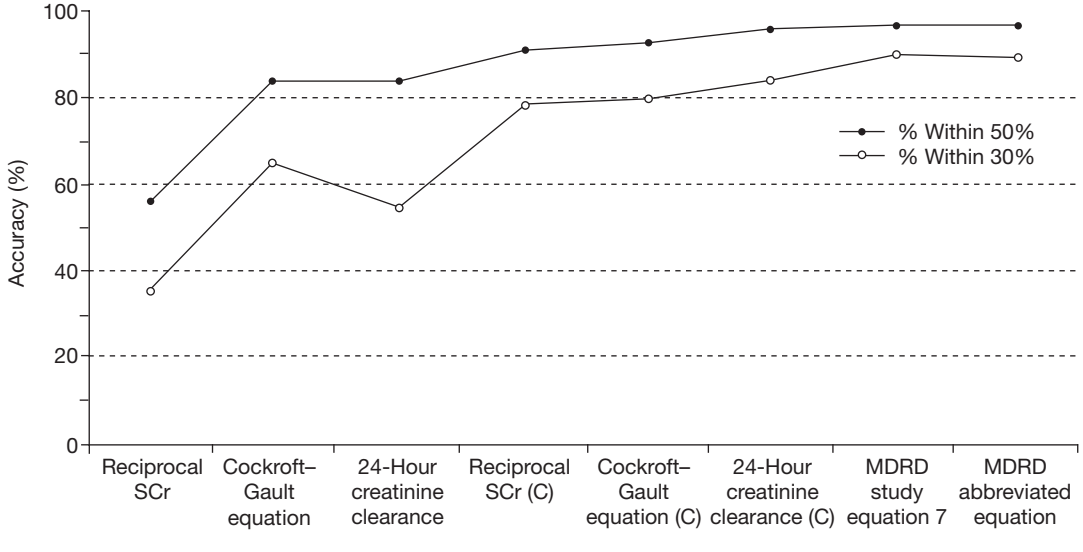

Figure 2.1 Accuracy of different estimates of GFR in adults, expressed as the percentage of estimates within 30% and 50% of the measured GFR in the MDRD Study validation sample (n = 558). Estimates denoted with [C] include a calibration correction of 0.69 for 100/serum creatinine, 0.84 for Cockcroft–Gault equation, and 0.81 for 24-hour creatinine clearance to show performance after bias is eliminated using a multiplicative correction factor. Analysis of MDRD study[14] data prepared by Tom Greene, Ph.D. With permission from reference 1.

major potential errors that limit the accuracy of creatinine clearance: (a) incomplete urine collection, (b) increasing tubular secretion of creatinine – especially with falling GFR – which may overestimate the true GFR. During the K/DOQI process, the investigators examined the accuracy of SCr, creatinine clearance, and the predictive equations vs measured I^{125} iothalamate GFR from the MDRD study cohort.[1] Results from that evaluation can be seen in Figure 2.1. Predictive equations were more reliable estimates of GFR than either SCr or creatinine clearance. Indeed, the day-to-day coefficient of variation of creatinine clearance has been reported as high as 27% by some investigators.[19] The K/DOQI guidelines recommend that a 24-hour urine for creatinine clearance is no longer suggested as an estimate of GFR.

Cystatin C

Given the limitations of SCr, serum cystatin C has been proposed as a screening test in an attempt to improve the detection of a reduction in GFR.[20] Cystatin C is a member of the family of cysteine proteinase inhibitors. It has a low molecular weight (13 kDa) and is produced at a constant rate by all nucleated cells. Cystatin C has been identified as a housekeeping gene whose constant production is independent of age, gender, muscle mass, etc. There are conflicting data as to whether its production may be variable in certain rare malignancies (e.g., metastatic melanoma or colon cancer). Cystatin C is freely filtered by the glomerulus, not secreted by the renal tubules, but is almost entirely reabsorbed and cannibalized by the proximal tubule. The latter characteristic negates the calculation of urinary clearance of cystatin C as a measure of GFR. Since it is completely filtered by the kidney, does not return to the bloodstream, and is not secreted by renal tubules, it has been proposed as an ideal endogenous marker of GFR.

The first radioimmunoassay (RIA) to quantify cystatin C in serum was developed in 1979. Subsequent methods to detect cystatin C were developed using radiofluorescent and enzymatic immunoassays. More recently, automated homogeneous immunoassays using latex or polystyrene particles coated with cystatin C antibodies have been developed and FDA approved.[21,22]

Multiple studies have validated the use of cystatin C as a 'renal marker' in adults, as serum cystatin C correlated with measurement of an impaired GFR. In a recent review, the authors analysed 24 studies that examined the utility of cystatin C versus SCr for detecting an impairment of GFR (usually GFR of less than 80 ml/min, range 60–90 ml/min).[20] Fifteen studies concluded that cystatin C was superior to SCr and nine studies suggested equivalence. In aggregate, these studies consistently demonstrated that cystatin C performed at least as well as SCr as a 'renal marker in adults, in pediatric patients above the age of four, and in selected renal transplant patients'. Importantly, it must be recognized that these initial studies of cystatin C as a 'marker' of GFR were generally trying to distinguish between 'normal' GFR (greater than 80 ml/min) versus 'impaired' GFR. Cystatin C often had a better diagnostic sensitivity, specificity, negative predictive value and ROC curves than SCr in an adult population for identifying a patient with impaired GFR.[23] However, measurement of serum cystatin C as a *direct* quantification or estimate of GFR has not been well studied. The utility of cystatin C in formulating prediction equations – especially in patients with GFRs greater than 60 – is currently under investigation. Overall, the recent literature suggests that cystatin C may have a role in assessing kidney function in selected patient groups for whom the disadvantages of SCr have become apparent.

Prediction equations

The overall goal of the NKF's K/DOQI Clinical Practice Guidelines was to develop a standard definition and staging of CKD to assist the clinician in early recognition and treatment of CKD and its complications. The Clinical Practice Guidelines recommend GFR as the keystone for the definition and staging of CKD. Given the limitations of SCr and creatinine clearance, the K/DOQI guidelines recommended predictive equations for the estimation (not precise measurement) of GFR based upon SCr measurements. In children, the Schwartz[24] and Counahan–Baratt[25] equations are recommended. For adults the guidelines recommend two formulae: (1) Cockroft–Gault equation, and (2) the abbreviated MDRD study equation. The equations are defined as follows:

Cockroft–Gault:[26]
CCr (ml/min) = (140 – age) × lean body weight (kg)/pCr (mg/dl) × 72

MDRD abbreviated formula:[1]
GFR (ml/min/1.73 m^2) = 186.3 × ((SCr) exp [–1.154]) × (age exp [–0.203]) × (0.762 if female) × (1.180 if African American)

Given the greater likelihood of the MDRD equation predicting iothalamate measured GFR, the guidelines favoured the use of the abbreviated MDRD equation in the clinical practice of adult medicine (see Figure 2.1). K/DOQI authors defined the 'accuracy' of eGFR as the percentage of eGFR estimates within 30% of the measured iothalamate GFR (iGFR). In adults, the abbreviated MDRD formula performed better than the Cockcroft–Gault (CG) equation with only 10% of the eGFR falling outside of the 30% accuracy range.

It is critical to appreciate the limitations of the eGFR derived from the predictive equation. First and foremost, one must recognize that these predictive equations are creatinine-based *estimates* (not measurements) of GFR recommended