Assessment of the glomerular filtration rate in children: methodological aspects

BEPALING VAN DE GLOMERULAIRE FILTRATIE SNELHEID BIJ KINDEREN: METHODOLOGISCHE ASPECTEN

Proefschrift

ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 11 januari 2006 om 13.45 uur

door LYONNE KARIN VAN ROSSUM

geboren te Nijmegen

PROMOTIECOMMISSIE

PROMOTIECOMMISSIE

Promotor:

Prof.dr. A.G. Vulto

Overige leden:

Prof.dr. A.J. van der Heijden

Prof.dr. J. Lindemans

Prof.dr. L.A.H. Monnens

The printing of this thesis was financially supported by

Voor mama

CONTENTS

8 Introduction

10 Chapter 1
Determination of the glomerular filtration rate in children

40 Chapter 2
Optimal sampling strategies to assess inulin clearance in children by the inulin single injection method

64 Chapter 3
Determination of inulin clearance by single injection or infusion in children

76 Chapter 4
Bepaling van de glomerulaire filtratiesnelheid bij kinderen door middel van de inuline single injectie methode

90 Chapter 5
Estimation of the glomerular filtration rate in children: which algorithm should be used?

108 Chapter 6
Renal extraction of cystatin C versus ^{125}I-iothalamate in hypertensive patients

118 Chapter 7
Discussion

128 Chapter 8
Summary

132 Samenvatting voor niet ingewijden
138 List of publications
140 Dankwoord

INTRODUCTION

Renal function tests are important to evaluate the progression of kidney disease and to monitor renal function during drug treatment. For the assessment of renal function the mechanisms responsible for the formation of urine, namely glomerular filtration, tubular reabsorption and tubular secretion, must be taken into consideration. The excretion of endogenous and exogenous substances is mainly regulated by glomerular filtration. Glomerular filtration rate (GFR) is considered the most important parameter to evaluate renal function and it is affected by most kidney diseases.

For children's convenience it is important that the test to determine GFR can be performed in a short time window with minimal intervention (the total number of blood samples required and the blood volume at each sampling time have to be small). On the other hand it is important that the test shows a high accuracy. In general, tests that are most accurate are also those that are most elaborate and costly. Traditional accurate determination of GFR requires a lot of blood samples or a precise collection of urine, which is difficult in children.

The aim of this thesis was to develop a simple, practical, convenient and accurate method to determine the glomerular filtration rate adapted to the specific requirements of children.

CHAPTER 1

DETERMINATION OF THE GLOMERULAR FILTRATION RATE IN CHILDREN

see pages 30 - 39 for references

Glomerular Filtration Rate (GFR) is considered the most fundamental parameter to evaluate renal function in suspected renal diseases and to monitore renal function during treatment. The ideal marker for the determination of GFR is physiologically inert, freely filtered in the glomerulus and neither secreted, reabsorbed, synthesized, nor metabolized by the kidney. In addition, in case of an endogenous marker, a constant production rate is required.
Several markers can be used for the determination of GFR: creatinine and cystatin C as endogenous marker, and inulin, radio-isotopes (^{125}I-iothalamate, ^{51}Cr-EDTA and ^{99m}Tc-DTPA) and iohexol as exogenous marker. Inulin, a polysaccharide, has all of the properties of an ideal marker and is therefore considered the gold standard marker [1].
The classic method to determine the inulin clearance requires an intravenous infusion of inulin and timed urine collections over a period of several hours, which makes it both costly and cumbersome. As a result a number of alternative methods and markers for the determination of GFR has been developed.

In this chapter the pros and cons of several markers for the determination of GFR especially in children will be described. Some years ago Rahn et al. published a review about how to assess glomerular function in human subjects [2]. Gaspari et al. has described the usefulness of several markers for the determination of the glomerular filtration rate, particularly the marker iohexol [3]. However, those papers did not specifically refer to children and in recent years new markers for GFR (such as cystatin C) have been proposed. There are no methods specially designed for children, most methods used for the determination of GFR have been derived from adults. However, some markers for GFR are more preferable in children. In particular, special formulae to estimate GFR based on creatinine concentration have been developed for children.
Finally it will become apparent which methods are accurate and practical in order to determine the GFR in children in either daily practice or in a clinical research setting.

METHODS FOR THE DETERMINATION OF GFR

GFR can be determined by measuring the appearance of a marker in the urine (urinary clearance) or the disappearance of the marker from the blood (plasma clearance).

Urinary clearance

The urinary clearance of a marker is defined as the volume of plasma from which the marker would have to be totally cleared to account for its excretion in the urine during a certain period of time. It always requires the measurement of the urinary excretion rate. The urinary clearance is calculated by dividing the urinary excretion rate of the marker by its plasma concentration (Table I). Both endogenous and exogenous markers can be used for the determination of the urinary clearance. For endogenous markers 3 to 4 accurately timed urine samples are collected [1]. The exogenous marker is administered by continuous intravenous infusion until a steady state concentration has been reached. After the equilibration period also 3 to 4 accurately timed urine samples are collected [1]. During the equilibration and the

Table I. Methods for determination of GFR

Clearance	Type of marker	Method	Urine collection
Urinary clearance	endogenous	-	yes
	exogenous	continuous infusion	yes
	exogenous	single injection	yes
Plasma clearance	exogenous	continuous infusion	no
	exogenous	single injection	no

GFR can be determined by measuring the excretion of a marker in the urine (urinary clearance) or the disappearance of the marker from the blood (plasma clearance). For determination of the urinary clearance both endogenous and exogenous markers for GFR can be used, while for determination of the plasma clearance only exogenous markers can be applied.

period of urine collection diuresis is induced to maintain a high urine flow rate. Critical aspects of this method are the required time for equilibration of the exogenous filtration marker in body fluids, an adequate urine flow rate for complete emptying of the bladder, an accurate measurement of the urine volume for each clearance period and a precise recording of the duration of the urine collection period. In infants and young children collection of timed urinary specimens is difficult and requires bladder catheterization.

Plasma clearance

Instead of measuring the excretion of a marker in the urine, one can also measure the rate of disappearance of the marker from the plasma, which is indicated as the plasma clearance. This method can only be performed with an exogenous marker. The plasma clearance of an exogenous marker can be measured by use of either a continuous intravenous infusion or a single bolus injection. The continuous infusion method is based on the concept that when the plasma concentration of the marker is constant (steady state concentration) and the volume of distribution is saturated with the marker (state of equilibration), the rate of excretion equals the rate of infusion [1, 4]. GFR can be calculated from the infusion rate, the concentration of the marker in the infusate and the plasma concentration of the marker

Plasma sampling	Calculation of GFR (mL/min)
yes	$\frac{U \times V}{P}$
yes	$\frac{U \times V}{P}$
yes	$\frac{U \times V}{P}$
yes	$\frac{I \times R}{P}$
yes	$\frac{D}{AUC}$

U: concentration of the marker in urine (mg/mL); V: urine flow rate (mL/min); P: concentration of the marker in plasma (mg/mL); I: concentration of the marker in infusate (mg/mL); R: infusion rate (mL/min); D: dose (mg); AUC: Area Under the Curve (min*mg/mL)

(Table I). The time to reach a steady state concentration of the marker is critical. GFR is overestimated if a steady state concentration has not yet been reached.
In general the steady state concentration of a drug (90% of the steady state) is reached in 3.3 half-lives [5]. A loading dose can be given to reach the steady state concentration more rapidly. Calculation of an appropriate infusion rate and its matching loading dose is important for reaching equilibration in a short time [6].
With the single injection method, a bolus injection of the marker is administered and blood samples are collected to construct a plasma concentration-time-decay-curve. The plasma clearance of the marker is calculated from the dose and the Area Under the concentration-time Curve (AUC), using a classical pharmacokinetic approach [1] (Table I). For practical and convenient application in children it is important that the number of blood samples is reduced to a minimum to construct the plasma concentration-time curve. Critical aspects of the single injection method are the pre-analytical process and the pharmacokinetic analysis. According to the pre-analytical process it is important to know the exact administered dose of the marker and to note the exact time of blood sampling. Several computer programs can be used for the pharmacokinetic analysis of the data, however one has to realize that the programs always produce a value and it is up to the operator to evaluate if this value is correct.

MARKERS FOR THE DETERMINATION OF GFR

1. Exogenous markers

INULIN

Inulin is a polymer of fructose with a molecular weight of about 5200 Da (Table II) and has all of the properties of an ideal filtration marker. The suitability of inulin as a marker for GFR has been described by Smith in 1951 [7]. Because of the poor solubility of inulin and the need to heat it before administration, an inulin analogue has been developed. This analogue, called polyfructosan-S (sinistrin, Inutest®), is more soluble and has a molecular weight of about 3500 Da. The clearance and the volume of distribution of this inulin analogue do not differ from that of inulin [8].
Inulin can be analyzed in urine and plasma by a classical colorimetric reaction (anthrone method) [9], or by an enzymatic method [10-13]. Both methods are based on the hydrolysis of inulin to fructose. Glucose and other carbohydrates interfere with the anthrone method and have to be removed before analysis. The enzymatic assay shows less interference.

see pages 30 - 39 for references

URINARY CLEARANCE OF INULIN

The urinary clearance of inulin is regarded to be the gold standard for the determination of GFR. Due to the disadvantages mentioned before this method is not commonly applied in clinical practice in children.

PLASMA CLEARANCE OF INULIN

A high degree of correlation between the plasma clearance by continuous infusion and the urinary clearance of inulin in children was found [14-16]. The time to reach a steady state concentration is critical for the continuous infusion method. An equilibration period of one to three hours is commonly used but there are data indicating that a period of more than 12 hours may be required for complete equilibration of inulin in the extra cellular space [15]. In newborns a 24-hour infusion of inulin is necessary since they have a low GFR (reference value for GFR in newborns < 2 weeks: 25 - 35 mL/min/1.73m²) and a relatively large extra cellular fluid compartment [17, 18].

The inulin single injection method is based on the pharmacokinetic concept that the clearance can be calculated from the dose and the AUC as explained before (Table I). For the determination of the inulin clearance with the single injection method, a bolus injection of inulin is administered and blood samples are collected up to 180 - 240 min after injection. Usually a two-compartment model is applied to construct the plasma concentration-time curve, but a three-compartment model has also been proposed [19, 20].

In two published studies, the plasma clearance by the single injection method was compared with the urinary clearance of inulin in children [21, 22]. A good correlation between the GFR determined by the single injection method and the GFR measured by means of the urinary clearance was found (r=0.82), however the number of children studied was small (n=13) [22].

We compared the single injection method with the continuous infusion method to determine the plasma clearance of inulin in 24 children. For the single injection method 5000 mg/m² of inulin was administered as bolus injection and blood samples were drawn at 10, 30, 90 and 240 minutes after administration. For the continuous infusion method inulin was started overnight and blood samples were collected the next day. The inulin plasma clearance determined by the single injection method was on average 9.7 mL/min/1.73m² higher than the clearance determined with the continuous infusion method (95% CI: 5.3; 14.2). The difference between both methods was smaller at lower GFR [23].

For practical and convenient application in children it is important that the number of blood samples is reduced to a minimum to construct the required plasma concentration-time curve. A reduction of the total number of blood samples from 12 to 7 in children has been published [24]. We described optimal sampling strategies for the inulin single injection method with at least 1 blood sample in children [25].

RADIO-ISOTOPES

A number of radionuclide-labeled compounds, including ^{125}I-iothalamate, ^{51}Cr-EthyleneDiamineTetra-Acetic acid (^{51}Cr-EDTA) and ^{99m}Tc-DiethyleneThiaminePenta-Acetic acid (^{99m}Tc-DTPA), can be used to determine GFR (Table II). The advantage of using radionuclides as marker for GFR is that they are easy to administer, can be measured with great accuracy, and are relatively cheap. Drawbacks related to radiation exposure and safety have led to a limited use of these markers in children. Especially the use of infusion of radionuclides for several hours is undesirable and therefore for these markers single injection methods have been developed, as well.

Table II. Markers for the determination of GFR

Type of marker	Marker	Molecular weight (Da)
exogenous	inulin	5200
	$^{125 \text{ or } 131}$I-iothalamate	614 (iothalamate)
	^{51}Cr-EDTA	292 (EDTA)
	^{99m}Tc-DTPA	393 (DTPA)
	iohexol	821
endogenous	creatinine	113
	cystatin C	13300

see pages 30 - 39 for references

IOTHALAMATE

The marker iothalamate has a molecular weight of 614 Da and is labeled with ^{125}Iodine or ^{131}Iodine. Both urinary and plasma clearance of labeled iothalamate can be determined. The plasma clearance of ^{125}I-iothalamate with single injection of the marker correlated closely with the urinary clearance of inulin in children, however an overestimation of on average 12% was found and the difference between the methods increased with decreasing GFR [26]. Possibly the total sampling period of 60 minutes for the ^{125}I-iothalamate single injection method was too short. The urinary clearance of ^{131}I-iothalamate agreed well with the urinary clearance of inulin in a small group of children (r=0.995; 30 studies in 10 children) and had a small standard error [27]. This result is in contrast with the observation of Rahn et al. that the urinary clearance of iothalamate in adults exceeded the urinary clearance of inulin, which suggested that tubular secretion of iothalamate occurs [2]. It is unclear whether these conflicting results can be explained by differences in age or have other reasons.
A single injection method with subcutaneous administration of the marker was developed for the determination of the urinary clearance of ^{125}I-iothalamate in children and had a mean coefficient of variation of less than 15% [28].

Unlabeled iothalamate can also be used for determination of GFR. The concentration of unlabeled iothalamate in serum and urine is measured by High Performance Liquid Chromotography (HPCL) or X-ray fluorescence. Mak et al. reported that there was no significant difference between the plasma and urinary clearance of unlabeled iothalamate when both were measured simultaneously in children with a renal transplant [16]. Sharma et al. showed that there was no difference between the plasma clearance of subcutaneously infused unlabeled iothalamate and the urinary clearance of ^{125}I-iothalamate in children and young adults [29]. A mean ratio (plasma clearance / urinary clearance) of 0.99 (limits of agreement 0.83-1.23) was found.

EDTA

Several studies have been reported on the use of EDTA (molecular weight 292 Da) labeled with ^{51}Cr for the determination of GFR in children [30-43]. In most cases the plasma clearance of ^{51}Cr-EDTA with single injection of the marker was determined. The results from studies comparing the ^{51}Cr-EDTA clearance with the clearance of other markers in children are presented in Table III. The plasma clearance of ^{51}Cr-EDTA with single injection agreed well with the plasma clearance of inulin if a two-compartment analysis was applied. With one-compartment analysis the AUC was underestimated and as a consequence the clearance was overestimated.
Optimalisation of the single injection method for ^{51}Cr-EDTA in children has been described by several authors and resulted in reduction of the required number of blood samples to even 1 sample [31, 36, 39, 40]. It is possible to use capillary instead of venous blood samples for

Table III. Results of studies comparing the ^{51}Cr-EDTA clearance with a reference clearance in children

Author	n	Population	Reference method
Aperia et al. [30]		patients with renal diseases	plasma clearance of inulin with single injection
	37 21		
Gibb et al. [37]	11	diabetic patients	urinary clearance of inulin with continuous infusion
Mak et al. [16]	13 (15 tests)	patients with renal transplant	plasma clearance of inulin with continuous infusion
			plasma clearance of iothalamate with continuous infusion
Vögeli et al. [43]	28	patients with various GFR	urinary clearance of inulin with continuous infusion

see pages 30 - 39 for references

Method for determination of ^{51}Cr-EDTA clearance	Results	Range of GFR
plasma clearance with single injection		one patient with GFR < 50 mL/min/1.73m²
one-compartment analysis	significant overestimation	
two-compartment analysis	no significant differences	
urinary clearance with continuous infusion	underestimation with a mean of 7.9 mL/min/1.73m²	no patients with GFR < 50 mL/min/1.73m²
plasma clearance with single injection one-compartment analysis	mean ratio of 1.14 ± 0.09, not significant mean ratio of 1.15 ± 0.08, not significant	8 - 70 mL/min
plasma clearance with single injection compartment analysis not mentioned	good agreement (y = 0.991x)	8 - 212 mL/min/1.73m²

this method [33]. However, no comparison with the clearance of other markers (i.e. inulin) was made for these optimized methods and, in the case of application of a one-compartment analysis, it is very likely that the GFR is overestimated.

DTPA

^{99m}Tc-DTPA is a chelate of DTPA and ^{99m}Tc and commonly used in renal imaging. This compound can also be used to determine the GFR (Table II). The plasma clearance of ^{99m}Tc-DTPA determined by single injection agreed well with the plasma clearance of ^{51}Cr-EDTA in children with reflux nephropathy (n=154) [44]. Rodman et al. reported that binding of DTPA to serum compounds (i.e. proteins) is a confounding factor for the determination of the clearance [45]. In 17 children with cancer the plasma clearance of ^{99m}Tc-DTPA was determined with and without ultrafiltration of the serum samples. The median clearance of ^{99m}Tc-DTPA from unfiltrated serum was significantly lower than the clearance with ultrafiltrated serum. However, no comparison with a reference clearance (i.e. urinary clearance of inulin) was made. Methods based on renography have been described to determine the GFR using ^{99m}Tc-DTPA with the percentage renal uptake or the slope from the renogram as predictors for GFR [46-52]. The advantage of these methods is that no blood samples are required and that additional information regarding the anatomy of renal function can be obtained.

IOHEXOL

Since the use of radiolabeled markers for GFR in children is limited, new unlabeled contrast media have been introduced to determine GFR. Iohexol is a low osmolar non-ionic contrast medium, which is used for angiography and urography. It has a molecular weight of 821 Da, is distributed in the extracellular volume [53] and eliminated from plasma by glomerular filtration [54]. Iohexol can be analyzed in urine and plasma by HPCL [54] or by X-ray fluorescence [55]. The HPLC-analysis of iohexol is complex, since there are two isomers of iohexol with different retention times [55, 56]. For practical purpose usually the plasma concentration of one iohexol isomer is used for calculation of GFR.
A single bolus injection method with drawing one blood sample and applying an empirical formula for the distribution volume of iohexol has been developed for the determination of the plasma clearance of iohexol in infants and children [53, 57]. Usually a two-compartment model is used to construct the plasma concentration-time curve for iohexol, but in several studies a one-compartment model with a

see pages 30 - 39 for references

correction factor for the overestimation of GFR has been applied [53, 58] and even a three-compartment model has been suggested [59]. Lindblad et al. compared the plasma clearance of iohexol with the plasma (n=20) and urinary (n=54) clearance of inulin in children and young adults [60]. A correlation coefficient of 0.81 and 0.86, respectively was found. The agreement between the plasma clearance of iohexol and ^{99m}Tc-DTPA was investigated in infants and children [57, 58, 61]. The plasma clearance of iohexol was not significantly different from the plasma clearance of ^{99m}Tc-DTPA.

Stake et al. studied the effect of iohexol on renal function in 10 children [58]. No change in GFR was detected after administration of a standard dose of iohexol, however the number of patients in the study was small and the effect of iohexol on renal function was studied only at the day of administration.

2. Endogenous markers

CREATININE

Creatinine has a molecular weight of 113 Da and is a metabolic product of creatine and phosphocreatine in the muscle. Its production is proportional to the total muscle mass.
This leads to a variation in serum creatinine concentration across age, gender, race, nutritional status and body composition. Creatinine is not an ideal marker for GFR because it is not only freely filtered by the glomerulus, but also secreted by the proximal tubule. The extent of tubular secretion of creatinine is not constant and shows interpatient and intrapatient variability, which makes it impossible to use a constant correction factor for tubular secretion [62].
The proportion of total renal creatinine excretion due to tubular secretion increases with decreasing renal function. As a consequence the GFR based on the plasma creatinine concentration is overestimated, particularly at lower GFR. The tubular secretion of creatinine can be inhibited by several compounds (such as cimetidine), which are secreted by the same pathway [63-65].
The urinary clearance of creatinine can be determined or GFR can be estimated by a formula based on the plasma concentration of creatinine. The former method is not directly affected by differences in creatinine production. However, accurate collection of the required timed urinary samples is difficult. Simultaneous determination of the urinary clearance of creatinine and inulin in children showed a good agreement, but an overestimation of inulin clearance was found especially at low levels of GFR [66, 67]. This was to be expected, since tubular secretion of creatinine increases with decreasing renal function.

FORMULAE TO ESTIMATE GFR BASED ON CREATININE PLASMA CONCENTRATION

A number of formulae, including the reciprocal plasma concentration of creatinine, has been developed to estimate GFR. Variation in creatinine production due to age- and sex-related differences in muscle mass has been incorporated in these formulae. For adults

the formula of Cockroft and Gault is widely used [68]. However Paap et al. showed that this formula couldn't be applied in children [69]. The best-known formulae for children are the formula of Schwartz et al. [70] and the formula of Counahan et al. [71] (Table IV). Both formulae are based on a constant (k) multiplied by the child's body height (BH, cm) divided by the plasma creatinine concentration (Pcr, µmol/L):

$$GFR(mL/\min/1.73m^2) = \frac{k \times BH}{Pcr}$$

The difference between k in the formula of Schwartz (k = 48.7) and in the Counahan formula (k = 38) has been attributed to the use of different assays to measure the creatinine concentration and the difference in the reference method. Schwartz et al. used the urinary clearance of creatinine, while Counahan et al. applied the plasma clearance of ^{51}Cr-EDTA. Subsequently Schwartz et al. defined various values of k for infants and children of different ages, since the relationship between muscle mass and body height changes with age [72-74]. Morris et al. derived a formula closely related to the formula of Counahan (modified formula of Counahan with k = 40 for Pcr in µmol/L, Table IV) [75]. This formula is commonly used in clinical practice and inadvertently indicated as the 'Schwartz formula'.
Seikaly et al. reported that the GFR estimated by the formula of Schwartz overestimated the urinary clearance of ^{125}I-iothalamate (176 studies in 133 children; range of overestimation: 0.1 - 164%) and that this overestimation was larger with decreasing GFR [76]. This is not surprising since the formula of Schwartz is based on the creatinine clearance, which overestimates GFR especially at low GFR. Berg et al. reported that the GFR estimated by the formula according to Morris et al. was inaccurate to follow the renal function in pediatric patients with a renal transplant and treated with cyclosporine, especially during the first 2 years after transplantation [77]. This finding was most likely due to changes in habitus and increased creatinine secretion caused by cyclosporine.
It is important to realize that the formulae for GFR estimation should not be used in patients with body disproportions, reduced muscle mass or muscle diseases. In critically ill children of a pediatric intensive care unit the estimated GFR (formula of Schwartz) correlated significantly with the urinary clearance of creatinine (n=100). However, the discrepancy between the two methods was larger than 50% in 36 of 100 patients [78]. The most plausible reason for this finding was that many children in the pediatric intensive care unit had abnormally low muscle mass as a result of chronic disease or malnutrition.

see pages 30 - 39 for references

Skinner et al. studied the relationship between the GFR estimated by formulae (Schwartz, Counahan and Morris) and the plasma clearance of ^{51}Cr-EDTA in children with tumours, who were treated with chemotherapy [79]. They found extremely wide limits of agreement for all the three formulae. The bias was smallest for the formula according to Morris et al.

Léger et al. studied the relation between the plasma concentration of creatinine and the plasma clearance of ^{51}Cr-EDTA in children (n=64) and applied a population pharmacokinetic approach [80]. The derived algorithm to estimate the plasma clearance of ^{51}Cr-EDTA included body weight, square body height and plasma creatinine concentration (Table IV) and was tested in 33 children with kidney diseases. A good predictive performance was found.

Recently, Hellerstein et al. compared the formula of Léger et al. with the formula based on the ratio of the child's body height and the plasma creatinine concentration in 151 children [81]. The optimal value for k was determined locally (k = 44 for girls and boys younger than 13 years and k = 52 for boys older than 13 years). The formula of Léger et al. was not superior to the GFR calculated with k = 44 or 52. However, in the study of Hellerstein et al. the values for k were derived from and tested in the same data set, which is statistically incorrect.

We evaluated the predictive performance of several formulae by comparing them with the inulin plasma clearance (n=48, 5 - 18 years of

Table IV. Formulae to estimate GFR based on creatinine concentration

Author	Formula for estimation of GFR	Reference clearance
Counahan et al. [71][a]	$\frac{38 \times BH}{Pcr}$	plasma clearance of ^{51}Cr-EDTA
Léger et al. [80][b]	$\frac{56.7 \times Weight + 0.142 \times BH^2}{Pcr}$	plasma clearance of ^{51}Cr-EDTA
Morris et al. [75][a]	$\frac{40 \times BH}{Pcr}$	plasma clearance of ^{51}Cr-EDTA
Schwartz et al. [70][a]	$\frac{48.7 \times BH}{Pcr}$	urinary clearance of creatinine

BH: body height (cm); Pcr: plasma creatinine concentration (µmol/L)

[a]GFR in mL/min/1.73m²

[b]GFR in mL/min

age) [82]. The formulae with k= 40 (Morris) or k = 41.2 estimated GFR well (bias< 5%; precision: 25%). The formula according to Léger et al. overestimated the inulin plasma clearance.
One has to realize that the formulae to estimate GFR can only be applied for clinical daily practice. The formulae are too imprecise for an accurate determination of GFR as desired in a research setting. Pierrat et al. reported an overestimation of 20-25% for the formula according to Schwartz et al. [83]. We found that the difference between estimated GFR and inulin plasma clearance was larger than 10 mL/min/ 1.73m² in more than 40% of the patients [82]. Therefore the formulae cannot replace the classical method for determination of GFR.

CYSTATIN C
Cystatin C is a small cationic protein, which inhibits cysteine proteinase, and has a molecular weight of 13.3 kDa. Cystatin C is produced by all nucleated cells at a constant rate and is freely filtered by the glomerulus. Subsequently it is not secreted in the tubulus but mainly reabsorbed by tubular epithelial cells and catabolized completely [84, 85].
It has been reported that the plasma concentration of cystatin C was independent of gender [86, 87] and constant from 1 year of age [88-93]. However, Harmoinen et al. showed that children between 1 and 3 years in age had a slightly but significantly higher cystatin C plasma concentration than older children and proposed a reference interval for children from 3-16 years and younger children [94]. This was recently confirmed by Fischbach et al. [95]. Reference values for cystatin C in infants (< 1 year of age) have also been reported [90, 94, 96].
Elevated cystatin C concentrations have been reported for children with a renal transplant, which may be attributed to the use of (a large dose of) glucocorticoids [97-101]. The glucocorticoid-induced increase in cystatin C can probably be attributed to a promoter-mediated increase in transcription of the cystatin C gene. For this reason it has been suggested that specific reference intervals for patients on glucocorticoid therapy are needed [99, 100].
It has been reported that the concentration of cystatin C in patients with various types of cancer was increased, irrespective of renal function [102]. However, it was not quite clear whether the increase in cystatin C concentration was attributable to an increased production rate or to a decreased elimination [102, 103]. Furthermore, thyroid dysfunction has also an impact on plasma cystatin C concentration [104, 105]. A higher and a lower cystatin C concentration were found in hyperthyroid

and hypothyroid patients, respectively. A likely explanation for this founding is that the production rate of cystatin C is affected by thyroid dysfunction, possibly due to a metabolic-rate-mediated mechanism [105].

Several immunoassays have been developed for the analysis of cystatin C in plasma or serum. The most commonly used assays are the Particle-Enhanced Turbidimetric Immuno-Assay (PETIA) and the Particle-Enhanced Nephelometric Immuno-Assay (PENIA). These methods are based on the reaction between cystatin C antibodies and cystatin C in the sample. Cystatin C concentrations measured by PENIA seem to be slightly lower (about 30%) compared with PETIA [87, 94]. The use of different assays to measure cystatin C concentration and the lack of a standard method for calibration make a direct comparison between the methods difficult [87, 106].

The reciprocal of the plasma cystatin C concentration has been proposed as a parameter for GFR. The relationship between the reciprocal of the cystatin C concentration and the reference clearance in children are reported in Table V.
Several studies have been performed to evaluate the usefulness of cystatin C to estimate GFR [88, 91, 93, 107-114]. In general a better correlation was found for the reciprocal of plasma cystatin C concentration to the reference GFR than for the reciprocal of plasma creatinine concentration. However, in two papers a slightly lower correlation coefficient was reported [108, 109]. The finding of a higher correlation coefficient for cystatin C is not surprising, since the reciprocal of creatinine concentration alone is not the proper parameter for GFR. Filler et al. corrected the creatinine concentration for age and muscle mass and compared the correlation of the reciprocal of cystatin C concentration and the GFR estimated by the formula of Schwartz with the plasma clearance of radio-isotopes [93]. In that study a similar correlation coefficient was found for the reciprocal of cystatin C concentration compared with the GFR estimated by the formula of Schwartz and the areas under the receiver-operating characteristic curve were not significantly different.
An algorithm for cystatin C to estimate GFR was developed and tested in 101 children [88]. The mean difference between the methods was 2.37 mL/min/1.73 m² with + 46.4 and - 41.6 mL/min/1.73 m² as limits of agreement (mean difference ± 1.96 SD). The algorithm based on cystatin plasma concentration was not very accurate to estimate the GFR.
In an additional study GFR estimated by the algorithm for cystatin C was compared with the 24-hour creatinine clearance in children, who recently received a kidney transplant (n=24) [115]. The mean GFR for cystatin C was 40% below the value based on the 24-hour creatinine clearance, which could be explained by an increased concentration of cystatin C in patients with a renal transplant due to the use of glucocorticoids. Filler et al. derived a formula to estimate GFR from serum

Table V. Relationship of the reciprocal cystatin C concentration and the reference clearance in children

Author	Number of patients	Age (years)	Population patients with
Bökenkamp et al. [88]	83	11.2 ± 0.5 (mean ± sd)	various disorders
Filler et al. [89]	381	12.1 ± 4.8 (mean ± sd)	various renal disorders
Filler et al. [93]	225	11.2 ± 4.5 (mean ± sd)	various renal disorders
Filler et al. [107]	25 (28 tests)	12.8 ± 4.2 (mean ± sd)	renal transplant
Helin et al. [91]	69	1 - 16 (range)	not mentioned
Kilpatrick et al. [119]	64 (77 tests)	0.25 - 18 (range)	various disorders
Krieser et al. [108]	19 (28 tests)	8.35 - 19.1 (range)	renal transplant
Martini et al. [120]	99	1.0 - 17.9 (range)	various disorders
Samyn et al. [121]	62 (87 tests)	0.3 - 18.6 (range)	liver diseases
Stickle et al. [109]	67	1.8 - 18.8 (range)	various renal disorders
Willems et al. [110]	66	1.3 - 21.9 (range)	various renal disorders
Ylinen et al. [111]	52	2 - 16 (range)	various renal disorders

see pages 30 - 39 for references

Reference clearance	GFR (mL/min/1.73m²)	Method for analysis	Correlation (r)
inulin clearance	81.0 ± 4.5 (mean ± sd)	PETIA	0.88
plasma clearance of ^{51}Cr-EDTA	12 - 211 (range)	PETIA	0.64
plasma clearance of ^{51}Cr-EDTA (n=127) plasma clearance of ^{99m}Tc-DTPA (n=98)	not mentioned	PENIA	0.765
plasma clearance of ^{99m}Tc-DTPA	39 - 123 (range)	PENIA	0.85
plasma clearance of ^{51}Cr-EDTA	not mentioned	PETIA	0.83 (0.56 normal GFR, n=56) (0.75 reduced GFR, n=13)
plasma clearance of ^{51}Cr-EDTA	8 - 172 (range)	PENIA	0.81 (0.56 high GFR) (0.83 low GFR)
plasma clearance of ^{99m}Tc-DTPA	not mentioned	PETIA	0.76
urinary clearance of inulin	19 - 179 (range)	PETIA	0.64
plasma clearance of ^{51}Cr-EDTA	28 – 270 (range)	PENIA	0.78
plasma clearance of inulin	not mentioned	PETIA	0.77 (n=26, 4 - 12 years) 0.87 (n=34, 12 - 19 years)
plasma clearance of inulin	7 - 145 (range)	PENIA	0.937
plasma clearance of ^{51}Cr-EDTA	< 89 (n=19)	PETIA	0.89

cystatin C concentration and compared that formula with the formula of Schwartz (n=536; 1.0 – 18.0 years of age) [116]. The cystatin C-based formula showed a better performance than the formula of Schwartz, however it was not clear whether the formula was derived from and tested in the same data set.
Hellerstein et al. studied whether the ratio of urinary cystatin C to urinary creatinine could serve as parameter for GFR (n=82; 6.1 - 21.3 years of age) [117]. They concluded that the ratio was a reliable screening tool for detecting decreased GFR in children.
Recently, Podracka et al. compared the intra-individual variation of cystatin C and creatinine concentration in 20 children with an organ transplant [118]. The mean coefficient of variation was significantly lower for serum creatinine (7.7 ± 4.2%) than for cystatin C (10.3 ± 4.9%). However, after excluding patients with bladder augmentation the difference was no longer significant.

CONCLUSIONS

GFR is considered the most relevant parameter to evaluate renal function. Several markers are available to determine the GFR in children. It would be ideal if GFR could be determined accurately by a simple measurement of an endogenous marker, for example creatinine. However, creatinine is not an ideal filtration marker. Estimation of the GFR in children by a formula based on the creatinine concentration and the body height is commonly used in daily clinical practice. The formula according to Morris et al.with k = 40 showed a good predictive performance for rough estimation of GFR in children [75]. In general, the formulae based on plasma creatinine concentration provide an estimate of GFR that is accurate enough for general clinical purposes. However, it is important to realize that these formulae could not be used in patients with body disproportions, reduced muscle mass or muscle diseases.
Cystatin C, a recently proposed endogenous marker, may also be useful to estimate GFR in daily practice. An advantage of this marker is that the production rate of cystatin C is less altered by nonrenal factors than the production of creatinine. However, it can be concluded from the studies discussed herefore that cystatin C is not a better marker for GFR than creatinine. In addition, the measurement of cystatin C concentration is expensive and creatinine assays are already applied routinely for many years. Therefore the use of cystatin C in daily practice is limited.

see pages 30 - 39 for references

For the determination of GFR in a clinical research setting a more accurate method is required: the urinary or plasma clearance of an exogenous marker should be measured. Inulin, a polysaccharide, is regarded as the gold standard marker and the urinary clearance of inulin with continuous infusion as the standard reference method to determine GFR. Disadvantages of determination of the urinary clearance are that this method is time-consuming, inconvenient to the patient and that the collection of timed urinary specimens is difficult and requires bladder catheterization, especially in infants and young children. Determination of the plasma clearance of a marker is an alternative method. The plasma clearance of an exogenous marker can be determined by either continuous infusion or single injection of the marker.
In practice determination of the plasma clearance of inulin with continuous infusion is more complicated and time-consuming than the single injection method. The inulin single injection method becomes more practical and convenient since optimal sampling strategies have been developed. One should realize that the use of such strategies requires advanced pharmacokinetic software and correct interpretation.
In several papers alternative markers for GFR have been compared with inulin. The radiolabeled marker ^{51}Cr-EDTA has been investigated extensively in children. For this marker simplified methods with one blood sample have been described, however these methods have not been compared with the urinary or plasma clearance of inulin and no optimal sampling strategies have been developed. Although the plasma clearance of ^{51}Cr-EDTA with single injection is a good alternative for the plasma clearance of inulin, its use in children is restricted due to radiation exposure. The marker iohexol does not exhibit the disadvantage of radiation exposure, but the experience with this marker in children is limited. Furthermore, the analytical procedure is cumbersome.

In conclusion, estimation of GFR in children by a formula based on creatinine plasma concentration and body height can be used for daily practice. Inulin is, despite it is relatively expensive, still the marker of choice to determine GFR in children in a research setting. Determination of the plasma clearance of inulin is preferred to the urinary clearance, as timed urine collection in children is difficult. The single injection method with a sparse sampling scheme in combination with adequate pharmacokinetic software is equivalent to the more cumbersome continuous infusion method for determination of the inulin plasma clearance.

REFERENCES

1. Barratt TM, Avner ED, Harmon WE (1999) Pediatric Nephrology, 4th edn. Lippincott Williams & Wilkins, Baltimore, pp 351-352

2. Rahn KH, Heidenreich S, Bruckner D (1999) How to assess glomerular function and damage in humans. J Hypertens 17:309-317

3. Gaspari F, Perico N, Remuzzi G (1997) Measurement of glomerular filtration rate. Kidney Int 63:S151-154

4. Earle DP, Berliner RW (1946) A simplified clinical procedure for measurement of glomerular filtration rate and renal plasma flow. Proc Soc Exp Biol Med 62:262-264

5. Rowland M, Tozer T (1994) Clinical pharmacokinetics, concepts and application, 3rd edn. Williams & Wilkins, USA, pp 70-71

6. Zietse R, Blankestijn PJ, Pos B, Balk AH, Derkx FH, Weimar W, Schalekamp MA (1995) Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. Clin Nephrol 43:29-34

7. Smith HW (1951) The Kidney. Oxford University Press, New York, pp 231-238

8. Mertz DP (1963) Observations on the urinary clearance and the volume of distribution of polyfructosan-S, a new inulin-like substance. Experientia 19: 248-249

9. White RP, Samson FG, Kan L. (1954) Determination of inulin in plasma and urine by use of anthrone. J Lab Clin Med 43:475-477

10. Degenaar CP, Frenken LA, von Hooff JP (1987) Enzymatic method for determination of inulin. Clin Chem 33:1070-1071

11. Kuehnle HF, von Dahl K, Schmidt FH (1992) Fully enzymatic inulin determination in small volume samples without deproteinization. Nephron 62:104-107

12. Summerfield AL, Hortin GL, Smith CH, Wilhite TR, Landt M (1993) Automated enzymatic analysis of inulin. Clin Chem 39:2333-2337

13. Wong EC, Wilhite TR, Miller R, Smith CH, Landt M (1994) Less expensive enzymatic analysis for inulin with a kinetic assay. Clin Chem 40:1788-1789

14. Cole BR, Giangiacomo J, Ingelfinger JR, Robson AM (1972) Measurement of renal function without urine collection. A critical evaluation of the constant-infusion technic for determination of inulin and para-aminohippurate. N Engl J Med 287:1109-1114

15. Hellerstein S, Berenbom M, Alon U, Warady BA (1993) The urinary clearance and infusion clearance of inulin are similar, but not identical. Kidney Int 44: 1058-1061

16. Mak RH, Al Dahhan J, Azzopardi D, Bosque M, Chantler C, Haycock GB (1983) Measurement of glomerular filtration rate in children after renal transplantation. Kidney Int 23:410-413

17. Coulthard MG (1983) Comparison of methods of measuring renal function in preterm babies using inulin. J Pediatr 102:923-930

18. Van der Heijden AJ, Grose WF, Ambagtsheer JJ, Provoost AP, Wolff ED, Sauer PJ (1988) Glomerular filtration rate in the preterm infant: the relation to gestational and postnatal age. Eur J Pediatr 148:24-28

19. Odeh YK, Wang Z, Ruo TI, Wang T, Frederiksen MC, Pospisil PA, Atkinson AJ (1993) Simultaneous analysis of inulin and 15N2-urea kinetics in humans. Clin Pharmacol Ther 53:419-425

20. Orlando R, Floreani M, Padrini R, Palatini P (1998) Determination of inulin clearance by bolus intravenous injection in healthy subjects and ascitic patients: equivalence of systemic and urinary clearances as glomerular filtration markers. Br J Clin Pharmacol 46:605-609

21. Backlund L, Goransson M, Muller-Suur R, Olsen L (1983) Evaluation of glomerular filtration rate in infants. Acta Paediatr Scand Suppl 305:77-78

22. Muller-Suur R, Goransson M, Olsen L, Backlund G, Backlund L (1983) Inulin single injection clearance. Micro-sample technique useful in children for determination of glomerular filtration rate. Clin Physiol 3:19-27

23. Van Rossum LK, Cransberg K, De Rijke YB, Zietse R, Lindemans J, Vulto AG (2005). Determination of inulin clearance by single injection or infusion in children. Ped Nephrol 20:777-781

24. Swinkels DW, Hendriks JC, Nauta J, de Jong MC (2000) Glomerular filtration rate by single-injection inulin clearance: definition of a workable protocol for children. Ann Clin Biochem 37:60-66

25. Van Rossum LK, Mathôt RAA, Cransberg K, Vulto AG (2003). Optimal sampling strategies to assess inulin clearance in children using the inulin single injection method Clin Chem; 49:1170-1179

26. Silkalns GI, Jeck D, Earon J, Edelmann CM Jr, Chervu LR, Blaufox MD, Spitzer A (1973) Simultaneous measurement of glomerular filtration rate and renal plasma flow using plasma disappearance curves. J Pediatr 83:749-757

27. Cohen ML, Smith FG Jr, Mindell RS, Vernier RL (1969) A simple, reliable method of measuring glomerular filtration rate using single, low dose sodium iothalamate I-131. Pediatrics 43:407-415

28. Bajaj G, Alexander SR, Browne R, Sakarcan A, Seikaly MG (1996) 125Iodine-iothalamate clearance in children. A simple method to measure glomerular filtration. Pediatr Nephrol 10:25-28

29. Sharma AK, Mills MS, Grey VL, Drummond KN (1997) Infusion clearance of subcutaneous iothalamate versus standard urinary clearance. Pediatr Nephrol 11:711-713

30. Aperia A, Freyschuss U (1984) Comparison of plasma clearances of polyfructosan and 51Cr-EDTA in children. Acta Paediatr Scand 73:379-382

31. Brochner-Mortensen J, Haahr J, Christoffersen J (1974) A simple method for accurate assessment of the glomerular filtration rate in children. Scand J Clin Lab Invest 33:140-143

32. Brochner-Mortensen J, Rohbrandt K, Lauritzen RB (1977) Precision of single injection (51Cr)EDTA plasma clearance and endogenous creatinine clearance determinations in children. Scand J Clin Lab Invest 37:625-629

33. Brochner-Mortensen J, Christoffersen J (1977) Single injection (51Cr)EDTA plasma clearance determination in children using capillary blood samples. Scand J Clin Lab Invest 37:631-633

34. Chantler C, Barratt TM (1972) Estimation of glomerular filtration rate from plasma clearance of 51-chromium edetic acid. Arch Dis Child 47:613-617

35. Donath A (1971) The simultaneous determination in children of glomerular filtration rate and effective renal plasma flow by the single injection clearance technique. Acta Paediatr Scand 60:512-520

36. Fjeldborg P, Brochner-Mortensen J (1986) Determination of 51Cr-EDTA clearance in infants by a single capillary blood sample. Scand J Clin Lab Invest 46:335-340

37. Gibb DM, Dalton NR, Barratt MT (1989) Measurement of glomerular filtration rate in children with insulin-dependent diabetes mellitus. Clin Chim Acta 182:131-139

38. Groth S (1984) Calculation of 51Cr-EDTA clearance in children from the activity in one plasma sample by transformation of the biexponential plasma time-activity curve into a monoexponential with identical integral area below the time-activity curve. Clin Physiol 4:61-74

39. Groth S, Aasted M (1984) 51Cr-EDTA clearance determined by one plasma sample in children. Clin Physiol 4:75-83

40. Ham HR, Piepsz A (1991) Estimation of glomerular filtration rate in infants and in children using a single-plasma sample method. J Nucl Med 32:1294-1297

41. Ham HR, Piepsz A (1996) Feasibility of estimating glomerular filtration rate in children using single-sample adult technique. J Nucl Med 37:1805-1808

see pages 30 - 39 for references

42. Piepsz A, Pintelon H, Ham HR (1994) Estimation of normal chromium-51 ethylene diamine tetra-acetic acid clearance in children. Eur J Nucl Med 21:12-16

43. Vogeli B, Riedwyl H, Donath A, Oetliker O (1971) Comparison of glomerular filtration rate and effective renal plasma flow determinations obtained by a single injection technique and by means of a standard clearance technique in children. Acta Paediatr Scand 60:528-532

44. Griffiths PD, Drolc Z, Green A, Taylor CM, White RH (1988-89) Comparison of 51Cr-EDTA and 99mTc-DTPA slope clearances in children with vesicoureteric reflux. Child Nephrol Urol 9:283-285

45. Rodman JH, Maneval DC, Magill HL, Sunderland M (1993) Measurement of Tc-99m DTPA serum clearance for estimating glomerular filtration rate in children with cancer. Pharmacotherapy 13:10-16

46. Inoue Y, Ohtake T, Homma Y, Yoshikawa K, Nishikawa J, Sasaki Y (1998) Evaluation of glomerular filtration rate by camera-based method in both children and adults. J Nucl Med 39:1784-1788

47. Inoue Y, Yoshikawa K, Suzuki T, Katayama N, Yokoyama I, Kohsaka T, Tsukune Y, Ohtomo K (2000) Attenuation correction in evaluating renal function in children and adults by a camera-based method. J Nucl Med 41:823-829

48. Piepsz A, Denis R, Ham HR, Dobbeleir A, Schulman C, Erbsmann F (1978) A simple method for measuring separate glomerular filtration rate using a single injection of 99mTc-DTPA and the scintillation camera. J Pediatr 93:769-774

49. Rehling M, Jensen JJ, Scherling B, Egeblad M, Lonborg-Jensen H, Kanstrup I, Dige-Petersen H (1989) Evaluation of renal function and morphology in children by 99mTc-DTPA gamma camera renography. Acta Paediatr Scand 78:601-607

50. Shore RM, Koff SA, Hayes JR, Smith SP, Mentser M, Mahan JD (1986) Glomerular filtration rate in children: validation and longitudinal use of determination from the 99mTc DTPA renogram. Am J Kidney Dis 8:170-180

51. Shore RM, Koff SA, Mentser M, Hayes JR, Smith SP, Smith JP, Chesney RW (1984) Glomerular filtration rate in children: determination from the Tc-99m-DTPA renogram. Radiology 151:627-633

52. Yap HK, Sundram FX, Yip WC, Tay JS, Ang ES, Toh HJ, Joseph VT, Saw AH (1985) Estimation of glomerular filtration rate in children using 99m-technetium DTPA. A comparison with 51-chromium EDTA clearance. Acta Paediatr Scand 74:579-583

53. Stake G, Monclair T (1991) A single plasma sample method for estimation of the glomerular filtration rate in infants and children using iohexol, I: Establishment of a body weight-related formula for the distribution volume of iohexol. Scand J Clin Lab Invest 51:335-342

54. Krutzen E, Back SE, Nilsson-Ehle I, Nilsson-Ehle P (1984) Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. J Lab Clin Med 104:955-961

55. Gronberg T, Sjoberg S, Almen T, Golman K, Mattsson S (1983) Noninvasive estimation of kidney function by x-ray fluorescence analysis. Elimination rate and clearance of contrast media injected for urography in man. Invest Radiol 18:445-452

56. Gaspari F, Perico N, Ruggenenti P, Mosconi L, Amuchastegui CS, Guerini E, Daina E, Remuzzi G (1995) Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. J Am Soc Nephrol 6:257-263

57. Stake G, Monn E, Rootwelt K, Monclair T (1991) A single plasma sample method for estimation of the glomerular filtration rate in infants and children using iohexol, II: Establishment of the optimal plasma sampling time and a comparison with the 99mTc-DTPA method. Scand J Clin Lab Invest 51:343-348

58. Stake G, Monn E, Rootwelt K, Gronberg T, Monclair T (1990) Glomerular filtration rate estimated by X-ray fluorescence technique in children: comparison between the plasma disappearance of 99mTc-DTPA and iohexol after urography. Scand J Clin Lab Invest 50:161-167

59. Back SE, Krutzen E, Nilsson-Ehle P (1988) Contrast media as markers for glomerular filtration: a pharmacokinetic comparison of four agents. Scand J Clin Lab Invest 48:247-253

60. Lindblad HG, Berg UB (1994) Comparative evaluation of iohexol and inulin clearance for glomerular filtration rate determinations. Acta Paediatr 83:418-422

61. Stake G, Monn E, Rootwelt K, Monclair T (1991) The clearance of iohexol as a measure of the glomerular filtration rate in children with chronic renal failure. Scand J Clin Lab Invest 51:729-734

62. Smith HW (1951) The Kidney. Oxford University Press, New York, pp 182-194

63. Hellerstein S, Berenbom M, Alon US, Warady BA (1998) Creatinine clearance following cimetidine for estimation of glomerular filtration rate. Pediatr Nephrol 12:49-54

64. Hellerstein S, Simon SD, Berenbom M, Erwin P, Nickell E (2001) Creatinine excretion rates for urinary clearance studies. Pediatr Nephrol 16:637-643

65. Hellerstein S, Erwin P, Warady BA (2003) The cimetidine protocol: a convenient, accurate, and inexpensive way to measure glomerular filtration rate. Pediatr Nephrol 18:71-72

66. Arant BS Jr, Edelmann CM Jr, Spitzer A (1972) The congruence of creatinine and inulin clearances in children: use of the Technicon AutoAnalyzer. J Pediatr 81:559-561

see pages 30 - 39 for references

67. Hellerstein S, Alon U, Warady BA (1992) Creatinine for estimation of glomerular filtration rate. Pediatr Nephrol 6:507-511

68. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31-41

69. Paap CM, Nahata MC (1995) Prospective evaluation of ten methods for estimating creatinine clearance in children with varying degrees of renal dysfunction. J Clin Pharm Ther 20:67-73

70. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259-263

71. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM (1976) Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child 51:875-878

72. Schwartz GJ, Feld LG, Langford DJ (1984) A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr 104:849-854

73. Schwartz GJ, Brion LP, Spitzer A (1987) The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am 34:571-590

74. Schwartz GJ (1992) Does kL/PCr estimate GFR, or does GFR determine k? Pediatr Nephrol 6:512-515

75. Morris MC, Allanby CW, Toseland P, Haycock GB, Chantler C (1982) Evaluation of a height / plasma creatinine formula in the measurement of glomerular filtration rate. Arch Dis Child 57:611-615

76. Seikaly MG, Browne R, Bajaj G, Arant BS Jr (1996) Limitations to body length/serum creatinine ratio as an estimate of glomerular filtration in children. Pediatr Nephrol 10:709-711

77. Berg U (1991) Evaluation of the formula clearance as a measure of the glomerular filtration rate in cyclosporin-treated children following renal transplantation. Transpl Int 4:72-76

78. Fong J, Johnston S, Valentino T, Notterman D (1995) Length/serum creatinine ratio does not predict measured creatinine clearance in critically ill children. Clin Pharmacol Ther 58:192-197

79. Skinner R, Cole M, Pearson AD, Keir MJ, Price L, Wyllie RA, Coulthard MG, Craft AW (1994) Inaccuracy of glomerular filtration rate estimation from height/ plasma creatinine ratio. Arch Dis Child 70:387-390

80. Leger F, Bouissou F, Coulais Y, Tafani M, Chatelut E (2002) Estimation of glomerular filtration rate in children. Pediatr Nephrol 17:903-907

81. Hellerstein S, Berenbom M, DiMaggio S, Erwin P, Simon SD, Wilson N (2004) Comparison of two formulae for estimation of glomerular filtration rate in children. Pediatr Nephrol 19:780-4

82. Van Rossum LK, Mathot RA, Cransberg K, Zietse R, Vulto AG. Estimation of the glomerular filtration rate in children: which algorithm should be used? Ped Nephrol: in press

83. Pierrat A, Gravier E, Saunders C, Caira MV, Ait-Djafer Z, Legras B, Mallie JP (2003) Predicting GFR in children and adults: a comparison of the Cockcroft-Gault, Schwartz, and modification of diet in renal disease formulae. Kidney Int 64:1425-36

84. Grubb A (1992) Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 38:S20-27

85. Randers E, Erlandsen EJ (1999) Serum cystatin C as an endogenous marker of the renal function--a review. Clin Chem Lab Med 37:389-395

86. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Brodehl J (1998) Reference values for cystatin C serum concentrations in children. Pediatr Nephrol 12:125-129

87. Randers E, Krue S, Erlandsen EJ, Danielsen H, Hansen LG (1999) Reference interval for serum cystatin C in children. Clin Chem 45:1856-1858

88. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J (1998) Cystatin C--a new marker of glomerular filtration rate in children independent of age and height. Pediatrics 101:875-881

89. Filler G, Priem F, Vollmer I, Gellermann J, Jung K (1999) Diagnostic sensitivity of serum cystatin for impaired glomerular filtration rate. Pediatr Nephrol 13:501-505

90. Finney H, Newman DJ, Thakkar H, Fell JM, Price CP (2000) Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child 82:71-75

91. Helin I, Axenram M, Grubb A (1998) Serum cystatin C as a determinant of glomerular filtration rate in children. Clin Nephrol 49:221-225

92. Filler G, Witt I, Priem F, Ehrich JH, Jung K (1997) Are cystatin C and beta 2-microglobulin better markers than serum creatinine for prediction of a normal glomerular filtration rate in pediatric subjects? Clin Chem 43:1077-1078

93. Filler G, Priem F, Lepage N, Sinha P, Vollmer I, Clark H, Keely E, Matzinger M, Akbari A, Althaus H, Jung K (2002) Beta-trace protein, cystatin C, beta(2)-microglobulin, and creatinine compared for detecting impaired glomerular filtration rates in children. Clin Chem 48:729-736

94. Harmoinen A, Ylinen E, Ala-Houhala M, Janas M, Kaila M, Kouri T (2000) Reference intervals for cystatin C in pre- and full-term infants and children. Pediatr Nephrol 15:105-108

95. Fischbach M, Graff V, Terzic J, Bergere V, Oudet M, Hamel G (2002) Impact of age on reference values for serum concentration of cystatin C in children. Pediatr Nephrol 17:104-106

96. Montini G, Cosmo L, Amici G, Mussap M, Zacchello G (2001) Plasma cystatin C values and inulin clearances in premature neonates. Pediatr Nephrol 16:463-465

97. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J (1999) Cystatin C serum concentrations underestimate glomerular filtration rate in renal transplant recipients. Clin Chem 45:1866-1868

98. Bokenkamp A, van Wijk JA, Lentze MJ, Stoffel-Wagner B (2002) Effect of corticosteroid therapy on serum cystatin C and beta2-microglobulin concentrations. Clin Chem 48:1123-1126

99. Risch L, Herklotz R, Blumberg A, Huber AR (2001) Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. Clin Chem 47:2055-2059

100. Risch L, Huber AR (2002) Glucocorticoids and increased serum cystatin C concentrations. Clin Chim Acta 320:133-134

101. Risch L, Huber AR (2002) Serum cystatin C in transplantation. Kidney Int 61:1548

102. Kos J, Stabuc B, Cimerman N, Brunner N (1998) Serum cystatin C, a new marker of glomerular filtration rate, is increased during malignant progression. Clin Chem 44:2556-2557

103. Newman D (1999) More on cystatin C. Clin Chem 45:718-719

104. Fricker M, Wiesli P, Brandle M, Schwegler B, Schmid C (2003) Impact of thyroid dysfunction on serum cystatin C. Kidney Int 63:1944-1947

105. Jayagopal V, Keevil BG, Atkin SL, Jennings PE, Kilpatrick ES (2003) Paradoxical changes in cystatin C and serum creatinine in patients with hypo- and hyperthyroidism. Clin Chem 49:680-681

106. Bokenkamp A, Dieterich C, Schumann G (1999) Pediatric reference values for cystatin C revisited. Pediatr Nephrol 13:367-368

107. Filler G, Pham-Huy A (2002) Cystatin C should be measured in pediatric renal transplant patients! Pediatr Transplant 6:357-360

108. Krieser D, Rosenberg AR, Kainer G, Naidoo D (2002) The relationship between serum creatinine, serum cystatin C and glomerular filtration rate in pediatric renal transplant recipients: a pilot study. Pediatr Transplant 6:392-395

109. Stickle D, Cole B, Hock K, Hruska KA, Scott MG (1998) Correlation of plasma concentrations of cystatin C and creatinine to inulin clearance in a pediatric population. Clin Chem 44:1334-1338

110. Willems HL, Hilbrands LB, Van De Calseyde JF, Monnens LA, Swinkels DW (2003) Is serum cystatin C the marker of choice to predict glomerular filtration rate in paediatric patients? Ann Clin Biochem 40:60-64

111. Ylinen EA, Ala-Houhala M, Harmoinen AP, Knip M (1999) Cystatin C as a marker for glomerular filtration rate in pediatric patients. Pediatr Nephrol 13:506-509

112. Dharnidharka VR, Kwon C, Stevens G (2002) Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 40: 221-6

113. Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A (2005) Cystatin C as a marker of GFR--history, indications, and future research. Clin Biochem 38: 1-8

114. Laterza OF, Price CP, Scott MG (2002) Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem 48: 699-707

115. Bokenkamp A, Ozden N, Dieterich C, Schumann G, Ehrich JH, Brodehl J (1999) Cystatin C and creatinine after successful kidney transplantation in children. Clin Nephrol 52:371-376

116. Filler G, Lepage N (2003). Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? Pediatr Nephrol 18:981-5

117. Hellerstein S, Berenbom M, Erwin P, Wilson N, DiMaggio S (2004). The ratio of urinary cystatin C to urinary creatinine for detecting decreased GFR. Pediatr Nephrol 19:521-5

118. Podracka L, Feber J, Lepage N, Filler G (2005) Intra-individual variation of cystatin C and creatinine in pediatric solid organ transplant recipients. Pediatr Transplant 9:28-32

119. Kilpatrick ES, Keevil BG, Addison GM (2000) Does adjustment of GFR to extracellular fluid volume improve the clinical utility of cystatin C? Arch Dis Child 82:499-502

120. Martini S, Prevot A, Mosig D, Werner D, van Melle G, Guignard JP (2003) Glomerular filtration rate: measure creatinine and height rather than cystatin C! Acta Paediatr 92:1052-7

121. Samyn M, Cheeseman P, Bevis L, Taylor R, Samaroo B, Buxton-Thomas M, Heaton N, Rela M, Mieli-Vergani G, Dhawan A (2005) Cystatin C, an easy and reliable marker for assessment of renal dysfunction in children with liver disease and after liver transplantation. Liver Transpl 11:344-9

CHAPTER 2

OPTIMAL SAMPLING STRATEGIES TO ASSESS INULIN CLEARANCE IN CHILDREN BY THE INULIN SINGLE INJECTION METHOD

L.K. van Rossum[1], R.A.A. Mathot[1], K. Cransberg[2], A.G. Vulto[1]

[1] Department of Hospital Pharmacy,
[2] Department of Pediatric Nephrology,
Erasmus MC, Rotterdam, The Netherlands

Clinical Chemistry, 2003;49:1170-9

see pages 60 - 63 for references

ABSTRACT

Glomerular filtration rate in patients can be determined by estimating the plasma clearance of inulin with the single injection method. With this method a single bolus injection of inulin is administered and several blood samples are collected. For practical and convenient application of this method in children it is important that a minimal number of samples is drawn. The aim of this study was to develop and validate sampling strategies with a reduced number of samples for reliable prediction of inulin clearance in pediatric patients using the inulin single bolus injection method.

Complete inulin plasma concentration-time curves of 154 patients were divided in an index (n=100) and validation set (n=54). A population pharmacokinetic model was developed for the index set. Optimal sampling times were selected based on D-optimality theory. For the validation set Bayesian estimates of clearance were generated using the derived population parameters and concentrations at 2-4 sampling times. Bayesian estimates of clearance were compared with the individual reference values of clearance. The strategies with samples taken at 10 / 30 / 90 / 240 min, 10 / 30 / 240 min, 10 / 90 / 240 min, 30 / 90 / 240 min and 90 / 240 min produced a good prediction of inulin clearance (bias < 3% and not significantly different from zero, imprecision < 15%). Strategies with two to four samples including a sample at 240 min after administration of inulin produce an accurate prediction of inulin clearance in pediatric patients using the inulin single injection method. Even 1 blood sample at 240 min showed an acceptable performance. The proposed strategies are practical and convenient to children, and reduce repetitive blood sampling without compromising accuracy.

INTRODUCTION

Measurement of renal function, i.e. Glomerular Filtration Rate (GFR), is essential for evaluating suspected renal diseases and for studying changes in renal function in patients with renal failure. The ideal marker is freely filtered by the glomerulus, not reabsorbed, secreted or metabolized by the kidney, physiologically inert and does not alter renal function. Inulin, an exogenous marker, is such a marker and its renal clearance during continuous intravenous infusion is regarded as the gold standard for measuring GFR in children [1]. However, this method is complex, time consuming, invasive and requires urine collection. For these reasons the renal inulin clearance is not commonly used in clinical routine. An alternative method, which can be performed without collection of urine, is the determination of plasma clearance of inulin. The plasma clearance of inulin can be measured by use of either a continuous intravenous infusion or a single bolus injection. The former method is more accurate but time-consuming because a steady-state-situation has to be achieved [1]. With the latter method a bolus injection of inulin is administered and 10 to 12 serial blood samples are withdrawn for the construction of a plasma concentration-time-decay-curve. Adequate results have been reported using this method in adults [2, 3]. However, for practical and convenient application in children it is important that the number of blood samples is minimized.
The aim of this study was to design and validate an optimal sampling strategy with a reduced number of samples for reliable estimation of the inulin clearance in pediatric patients with renal disorders using the inulin single injection method.

METHODS

Patients

The development and validation of the optimal sampling strategies was performed using data, which were collected during routine determination of renal function in 154 pediatric patients treated at the University Hospitals of Rotterdam and Nijmegen, between June 1994 and June 1997. Twenty-six children were seen in the follow-up of a hemolytic uremic syndrome. The other children were known for impaired renal function due to a variety of renal and acquired diseases. Demographic parameters and pathophysiological characteristics of the patients are summarized in Table I.

see pages 60 - 63 for references

Table I. Patient characteristics of the full, index and validation set[1]

	Full data set	Index set	Validation set
Patients	154	100	54
male	96	63	33
female	58	37	21
Age (y)	9.0 (1.0-21.0)	10.0 (1.0-20.0)	6.5 (1.0-21.0)
Weight (kg)	26 (8-59)	28 (8-59)	23 (8-57)
Height (cm)	129 (73-183)	133 (73-183)	122 (74-169)
BSA (m^2)	0.98 (0.39-1.76)	1.03 (0.39-1.76)	0.89 (0.41-1.65)
BMI (kg/m^2)	16.2 (12.4-23.5)	16.6 (12.4-21.8)	15.7 (12.9-23.5)
Disorders			
HUS	26	17	9
other disorders	76	49	27
unknown	52	34	18
Center			
Nijmegen	99	65	34
Rotterdam	55	35	20
Inulin dose (mg)	2906 (1213-5074)	3031 (1213-5074)	2723 (1239-4769)
GFR[2] ($mL/min/1.73m^2$)	44.5 (2.9-138.8)	46.0 (5.5-138.8)	37.0 (2.9-102.0)

[1]Data are presented as median (range)

[2]GFR was calculated from serum creatinine and height according to Counahan et al (4).

BSA: body surface area; BMI: body mass index; HUS: hemolytic uremic syndrome

Study design

All patients had a single cannula into an antecubital vein. They received a single intravenous dose (5000 mg per 1.73 m² body surface area with a maximum of 5000 mg) of inulin (polyfructosan-S (Inutest®), Laevosan Gesellschaft, Linz, Austria) within one minute. Polyfructosan-S is an inulinlike polysaccharide. Serial blood samples (1 mL) were collected at 0, 10, 20, 30, 45, 65, 90, 120, 150, 180, 210 and 240 minutes after injection [5] to construct a concentration-time curve accurately [3]. Concentrations of inulin were measured in serum (0.1 mL) by an enzymatic method [6]. The coefficient of variation for this method was 0.6% at 481 mg/L and 0.4% at 995 mg/L [5]. The assay was linear in the range from 0 to 1720 mg/L.

Population pharmacokinetic analysis

The patients were randomly divided into an index data set (n=100) and a validation data set (n=54). Population pharmacokinetic models were developed independently for both data sets using the nonlinear mixed-effect modeling program NONMEM (double precision; version V, level 1.1, GloboMax LLC). External validation of the index set population model was performed by comparison of its pharmacokinetic parameters with those of the validation data set. The validation procedure and the 2:1 ratio of the index and validation data set are proposed in the FDA guideline on population pharmacokinetics [7].
Several pharmacokinetic models (eg. one-, two-, three-compartment models) were evaluated for description of the concentration time profiles of inulin. The first-order conditional estimation method was used throughout the analyses taking into account interaction between inter- and intra-individual variability. Pharmacokinetic parameters of the models were estimated in terms of clearance, central and peripheral volume of distribution and intercompartmental clearance (NONMEM code TRANS4). For instance, for a two-compartmental model the estimated parameters were clearance (CL), volume of distribution of the central compartment (V_1), intercompartmental clearance (Q) and volume of distribution of the peripheral compartment (V_2).
A proportional error model was used to describe the inter-individual variability. For instance, the variability in clearance was estimated using:

$$CL_i = CL_{pop} \times (1+\eta_i)$$

in which CL_i represents the CL of the ith individual, CL_{pop} is the typical value in the population and η is the inter-individual random variable with a mean of 0 and a variance of ω^2.

The residual intra-individual variability was modeled with a combined additive-proportional error model:

$$Cobs_{ij} = \varepsilon_{1,ij} + Cpred_{ij} \times (1+\varepsilon_{2,ij})$$

where $Cobs_{ij}$ is the jth observed concentration in the ith patient, $Cpred_{ij}$ is the predicted concentration, and ε_1 and ε_2 are independent random variables with a mean of 0 and a variance of σ_1^2 and σ_2^2. The factors ε_1 and ε_2 account for the difference between the observed and predicted concentration.
The population model was built step by step. At each step, a specific assumption was tested (e.g. one-compartment versus two-compartment model or correlation between clearance and body surface area). The main criterion of decision was the likelihood ratio test [8]. For hierarchical models the difference between their respective objective function values is approximately chi-squared distributed and, as a result, formal testing can be performed. The level of significance was set at $p<0.01$, corresponding to a difference of the objective function values of 6.6 points. The objective function value, which equals minus twice the logarithm of the likelihood of data, is a goodness-of-fit criterion provided by NONMEM and has no units. Secondary criteria were aspects of the various residual plots ("goodness-of-fit" plots) and the values of random-effects variances. Possible correlation between the demographic or pathophysiological indices and the pharmacokinetic parameters were explored by a three-step approach [9, 10].

In step 1, an initial NONMEM analysis provided the population pharmacokinetic parameters without taking into account the demographic factors. In step 2 the Bayesian parameter estimates of the individual subjects were plotted against the demographic factors of interest. From the scatter plots, demographic factors were selected that correlate with the pharmacokinetic parameters. In step 3, the NONMEM analysis was resumed, and the demographic factors selected in step 2 were entered into the NONMEM regression model in a stepwise manner to test if a significant correlation was present between the covariate and the pharmacokinetic parameter. Relationships between pharmacokinetic parameters and the following covariates were tested: AGE (years), body surface area (BSA, m²), body weight (WT, kg), height (HT, cm), body mass index (BMI, kg/m²), hemolytic uremic syndrome / other disorders (HUS / OD), center (CENTR, Rotterdam / Nijmegen) and SEX (male / female).
The covariates were introduced in the population pharmacokinetic model using linear relationships. For instance, the relationship between CL and BSA was modeled using:

$$CL = \theta_1 + \theta_2 \times (BSA - 1.1)$$

where θ_1 is the typical clearance of a patient with a body surface area of 1.1 m² and θ_2 is the increment of CL per m² BSA. Dichotomous variables were modeled as follows:

$$CL = \theta_1 \times \theta_2^{HUS}$$

where HUS is 0 or 1 (absence/presence of HUS),

θ_1 is the typical clearance of patients with other disorders and θ_2 is the fractional increase of CL in case of HUS.

The final population model was considered adequate when several criteria were met: (1) adequate fit of each individual concentration-time curve, (2) linear pattern of observed versus predicted inulin concentrations, (3) absence of trend in the weighted residuals versus time plot and (4) an approximately normal distribution of the weighted residuals. The graphical plots were created in Xpose 2.0, a S-Plus based model building aid (Mathsoft Inc, Seattle, USA) [11, 12].

Development of optimal sampling strategies

The population pharmacokinetic model based on the index data set was used for the development of the optimal sampling strategies whereas the validation data set (n=54) was used for validation of these strategies. Based on the population pharmacokinetic parameters derived from the index set optimal sampling times were selected by application of the D-optimality theory [13] as implemented in the software package Adapt II (release 4, 1997) [14]. The D-optimality theory minimizes the total overall variance of parameter estimates based on the Fisher information index.

The optimal sampling strategies were designed with 1 to 4 samples and a total sampling time of 90 to 240 minutes.

Validation of sampling strategies

Plasma concentration time data of the validation set were used to evaluate the optimal sampling strategies. Individual Bayesian estimates of clearance were calculated using the final population estimates of the index data set and the plasma concentrations at the optimal sampling times. The basis of Bayesian estimation is that for estimation of the individual pharmacokinetic parameters information from the population pharmacokinetic parameters is combined with information derived from the actual individual concentrations of the samples [15]. A weighted combination of individual and population information where the weighting depends on how much information the individual itself supplies, is applied. If a large number of accurate blood samples are available the Bayesian estimation will largely be determined by the concentration of those samples alone. If, in contrast, only 1 blood sample is available the Bayesian estimation will largely be determined by the information obtained from the population pharmacokinetic parameters. Bayesian analysis was performed using the POSTHOC option in NONMEM with

see pages 60 - 63 for references

MAXEVAL=0. The Individual reference value for clearance (reference inulin clearance) was obtained by fitting of the individual curves based on all blood samples using extended least squares estimation in NONMEM [16]. The predictive performance of the Bayesian estimates using the various sampling strategies was evaluated by calculating the mean relative prediction error (MPE%) and its 95% confidence interval as a measure of bias and the root mean squared relative prediction error (RMSE%) and its 95% confidence interval as a measure of imprecision [17]. MPE%, RMSE% and the standard error for MPE% were defined as follows:

$$MPE\% = \frac{\sum_{i=1}^{n} pe_i}{n} \times 100\%$$

$$SE\% = \sqrt{\frac{\sum_{i=1}^{n} (pe_i - MPE)^2}{n \times (n-1)}} \times 100\%$$

$$RMSE\% = \sqrt{\frac{\sum_{i=1}^{n} (pe_i)^2}{n}} \times 100\%$$

in which n is the number of clearance pairs (i.e., reference and predicted values) and pe is the relative prediction error (ln CL pred - ln CL ref). The relative prediction error was calculated with the natural logarithm of clearance to avoid bias in favour of high clearance values. Ninety five percent confidence interval of RMSE% were obtained by calculating 95% confidence interval of mean squared relative prediction error and extracting the root.

RESULTS

Population pharmacokinetic analysis

The full data set consisted of 1675 samples collected from 154 pediatric patients. Figure 1 shows all individual plasma concentration-time profiles of inulin. Patient characteristics of the index and validation set are summarized in Table I. Patients in the validation set were slightly younger and had a somewhat lower GFR.

Several compartmental models were evaluated for description of the inulin concentration-time profiles. The analysis was started with a one-compartment model with inter-individual variability estimated for both clearance and the volume of distribution. In this model a clear trend was visible in the plot of weighted residuals (WRES) versus time. This trend disappeared upon introduction of a peripheral compartment; the objective function decreased with 1388.0 points to a value of 10299.8 points ($p<0.001$). Fitting of a three-compartment model did not converge satisfactorily. With a two-compartment model interindividual variability could be estimated for all pharmacokinetic parameters. Estimates are given in Table II.

Individual empirical Bayesian estimates of the pharmacokinetic parameters were obtained from the basic model with no covariates included. Visual inspection of the plots of the covariates versus individual estimates of parameters indicated correlations between BSA, WT, HT and CL, V_1, Q and V_2.

For the categorical covariates the following

correlations were observed: CL and HUS and CENTR, V_1 and CENTR and V_2 and CENTR. BSA, WT and HT were highly correlated ($R^2 > 0.9$). Since inulin clearance is commonly normalized for an average BSA of 1.73 m² (units of GFR: mL/min/1.73 m²), BSA was selected as measure for body size in the population model. Stepwise introduction of relationships between all pharmacokinetic parameters and BSA in the population model reduced the objective function significantly with 158.9 (CL; $p<0.001$), 130.6 (V_1; $p<0.001$), 77.5 (Q; $p<0.001$) and 201.1 points (V_2; $p<0.001$) to a value of 9379.3 points (the objective function value of the basic model was 9947.4 points). Similar introduction of WT in the basic model produced an objective function of 9364.3 points. Despite the 15 points difference in favour of the weight-pharmacokinetic parameter relationships it was decided to continue with BSA. By introduction of the relationship between CL and BSA, the additive error converged to 0 and was omitted in the subsequent analyses. This indicated that the (residual) difference between observed and predicted concentrations could be described adequately with only a proportional error. Implementation of a relationship between HUS and CL resulted in the final population model (Table II). Implementation of CENTR in the population model did not produce significant reductions of the objective function.

Table III shows the regression equations for the pharmacokinetic parameters for the final model. The equations between BSA and the pharmacokinetic parameters

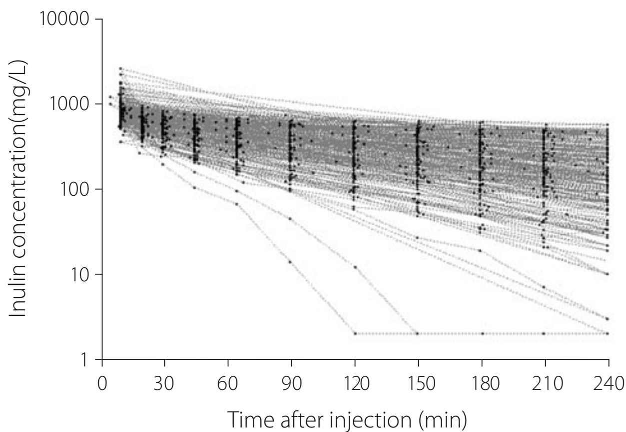


Figure 1

Individual plasma concentration-time profiles of inulin in pediatric patients (n=154) after administration of Inutest® (5000 mg per 1.73 m² BSA) within one minute.

see pages 60 - 63 for references

Table II. Estimates of the population pharmacokinetic parameters

	Index data set Basic model with two-compartments	Index data set Final model	Validation data set Final model
CL (mL/min)	30.6 (3.0)[1]	25.3 (1.9)	27.8 (2.6)
θ BSA (mL/min/m²)		32.9 (3.9)	31.4 (4.3)
θ HUS		2.46 (0.30)	2.67 (0.29)
V_1 (L)	1.79 (0.17)	2.07 (0.15)	2.39 (0.36)
θ BSA (L/m²)		1.70 (0.38)	2.77 (0.67)
Q (mL/min)	90.0 (5.5)	105 (4.0)	93.8 (8.0)
θ BSA (mL/min/m²)		104 (10)	85.4 (11.2)
V_2 (L)	3.15 (0.32)	3.58 (0.17)	3.45 (0.18)
θ BSA (L/m²)		3.90 (0.33)	3.12 (0.32)
Interindividual variability[2]			
CL (%)	150	56	64
V_1 (%)	73	56	50
Q (%)	42	21	18
V_2 (%)	47	29	27
Residual variability			
Additional error (mg/L)[3]	20.9 (3.8)	-	-
Proportional error (%)[4]	5.69 (1.33)	7.46 (0.64)	7.94 (0.84)

[1]data are presented as estimate (standard error) [2]coefficient of variation in population [3]expressed as absolute inulin concentration [4]expressed as percentage of inulin concentration

BSA: body surface area; HUS: hemolytic uremic syndrome

were rewritten in order to investigate whether the (intercompartmental) clearance (CL, Q) and volumes of distribution (V_1, V_2) could be expressed in units of mL/min/m² BSA and L/m² BSA, respectively. For instance, the relationship between CL and BSA was rewritten as follows: CL= intercept + slope x BSA. Fitting of this model to the data produced a goodness of fit comparable to the final model. Subsequently, the intercept was fixated to 0, thereby expressing clearance as CL per m² BSA and reducing the number of estimated parameters (only the slope is estimated). For all parameters population models were obtained which were significantly worse ($p<0.001$) than the models with intercepts included.

Inclusion of the covariate relationships reduced the unexplained inter-individual variability in CL, V_1, Q and V_2. For instance, variability in clearance was decreased from 150 to 56%: introduction of the covariates BSA and HUS explained 94% of the variability in CL. The goodness of fit of the final model is illustrated by plots of population and individual predicted concentration values versus observed inulin concentrations in the index set (Figure 2). The population predicted inulin concentrations are predicted from the population model without taking into account interpatient and residual variability (i.e. $\eta=0$ and $\varepsilon=0$). Individual predicted inulin concentrations are predicted by Bayesian estimation; for each patient individual η values are estimated based on individual concentrations. In the plot of population predicted versus observed concentrations a slight bias was present at high concentrations, especially at sampling time 10 min; the population model underpredicted the observations. Underprediction of observation could not be associated with weight, height, BSA or BMI of the patient; a pre-analytical error was plausible. The bias was absent in the plot of individually predicted versus observed concentrations, indicating an adequate fit of each individual concentration-time curve. For each individual in the index set pharmacokinetic parameters were estimated by Bayesian analysis. Median (and

Table III. Regression equations describing the relationship between the pharmacokinetic parameters and the covariates for the final model (index data set)

CL (mL/min)	$= (25.3 + 32.9 \times (BSA - 1.1)) \times 2.46^{HUS}$
V_1 (L)	$= 2.07 + 1.70 \times (BSA - 1.1)$
Q (mL/min)	$= 105 + 104 \times (BSA - 1.1)$
V_2 (L)	$= 3.58 + 3.90 \times (BSA - 1.1)$

see pages 60 - 63 for references

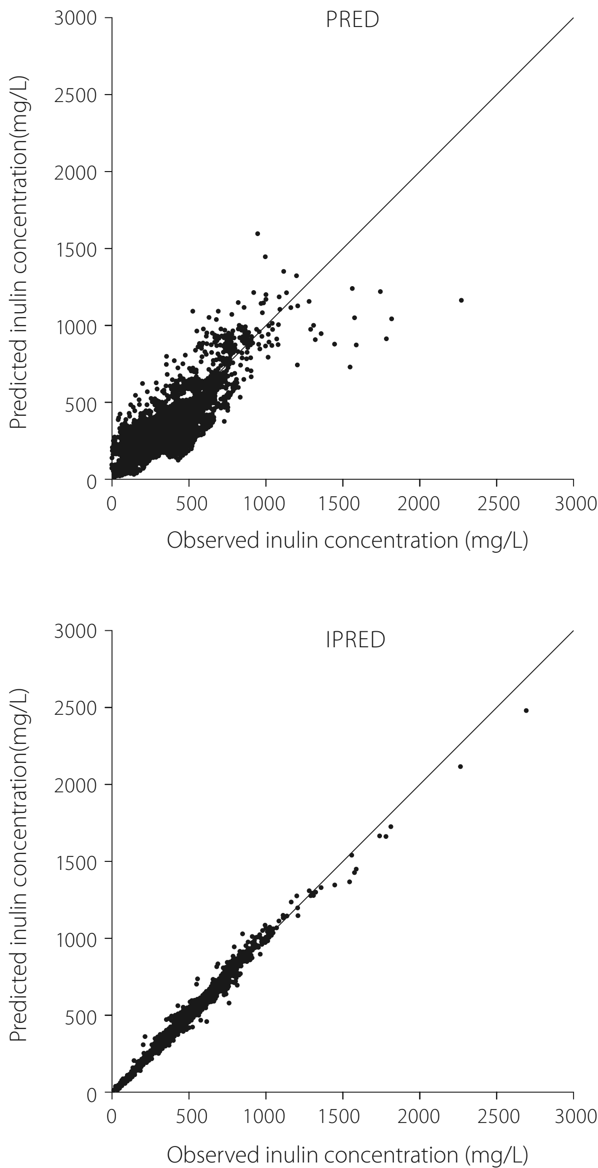


Figure 2

Population (PRED) and individual (IPRED) predicted versus observed inulin concentrations for the final population model of the index data set. Population predicted inulin concentration is based on the pharmacokinetic model without taking into account interpatient and residual variability (i.e. $\eta=0$ and $\varepsilon=0$). The individual predicted inulin concentration is predicted by Bayesian estimation. The solid line represents the line of unity.

range) of the individual estimates of CL, V_1, Q, V_2, distribution and elimination half-life were: 28 [2-123] mL/min, 1.93 [0.36-6.15] L, 94 [32-195] mL/min, 2.59 [0.84-8.71] L, 7.9 [3.6-12.3] min and 143 [30-918] min, respectively (n=100).
The developed population model was validated using the validation data set. No bias was observed when the predicted inulin concentrations based on the index population model were plotted against the observed concentrations (plot not shown). Independent development of a population model based on the validation

Table IV. Predictive performance of the inulin clearance for the sampling strategies in the validation data set

Strategy	Number of samples	Sampling times (min)	Bias MPE%	Bias 95% CI	Imprecision RMSE%	Imprecision 95% CI
1	4	10 / 30 / 90 / 240	1.8	[-1.1, 4.7]	10.7	[6.3, 13.8]
2	4	10 / 30 / 65 / 120	6.7	[-2.7, 16.1]	34.9	[0, 49.4]
3	3	10 / 30 / 90	0.5	[-18.4, 19.5]	68.9	[50.1, 83.6]
4	3	10 / 30 / 240	1.7	[-2.0, 5.6]	14.1	[4.8, 19.3]
5	3	10 / 90 / 240	2.6	[-0.5, 5.7]	11.7	[6.9, 15.0]
6	3	30 / 90 / 240	0.3	[-3.1, 3.7]	12.5	[8.8, 15.2]
7	2	10 / 240	1.7	[-2.5, 6.0]	15.7	[9.4, 20.1]
8	2	30 / 240	-0.1	[-4.7, 4.4]	16.4	[9.6, 21.2]
9	2	90 / 240	1.3	[-2.2, 4.8]	12.8	[9.1, 15.7]
10	1	240	1.0	[-4.0, 5.9]	18.2	[12.1, 22.7]
11	1	210	3.9	[-1.2, 9.0]	19.0	[12.3, 23.8]
12	1	180	3.3	[-5.9, 12.5]	33.6	[0, 49.8]
13	1	150	3.2	[-6.9, 13.3]	37.0	[0, 52.9]
14	1	120	6.8	[-4.0, 17.7]	40.1	[0, 57.1]

MPE: mean relative prediction error

RMSE: root mean squared relative prediction error

CI: confidence interval

see pages 60 - 63 for references

data set produced pharmacokinetic parameter estimates comparable to the index data set (Table II). Separate deletion of the 5 covariate-pharmacokinetic relationships from the model (CL vs BSA and HUS, V_1, Q and V_2 vs BSA) increased the objective function significantly ($p<0.001$). No other significant relationships were detected.

Development of optimal sampling strategies

For a typical patient with a body surface area of 1.1 m² the following four optimal sampling times were found in the observation period from 10 to 240 minutes: 10, 25, 79 and 240 min. Optimal sampling times were only marginally influenced by BSA and presence/absence of HUS. Samples close to the optimal sampling times were selected from the profiles in the validation set. Differences between theoretical and actual sampling times were within 15 minutes. The tested sampling strategies are summarized in Table IV.

Validation of sampling strategies

Individual reference values for the clearance and other pharmacokinetic parameters were calculated using the full plasma concentration-time profiles. Bias and imprecision of predicted clearance for the different sampling strategies are given in Table IV. None of the sampling strategies was significantly biased. Imprecision ranged from 10.7% to 68.9%. The sampling strategies 1 (10 / 30 / 90 / 240 min), 4 (10 / 30 / 240 min), 5 (10 / 90 / 240 min), 6 (30 / 90 / 240 min) and 9 (90 / 240 min) had a good predictive performance with a bias less than 3%, and an imprecision not exceeding 15%.

For example, Figure 3 shows a Bland-Altman plot of the Bayesian predicted clearance and the reference clearance for sampling strategy 1 (10 / 30 / 90 / 240 min) and 2 (10 / 30 / 65 / 120 min). Typically, in sampling strategy 2 a bias was present at low levels of inulin clearance; the predicted clearance was larger than the reference clearance. This can be explained by the fact that the elimination half-life is underestimated. This phenomenon was further investigated by stratification of predictive performance for an individual CL < 40 mL/min/1.73m² versus individual CL > 40 mL/min/1.73m². For CL > 40 mL/min/1.73m² no bias was observed and the imprecision was smaller than 15% for the sampling strategies 1 and 4-11.

For CL < 40 mL/min/1.73m² the clearance was slightly (but not significantly) overestimated with bias ranging from 2.8 – 4.8% for the sampling strategies 1 and 4-10. Imprecision was smaller than 15%. Strategy 1, for example, produced a bias of 2.8% (95% CI -0.5, 6.1%) and an imprecision of 9.8% (95% CI 0, 13.9%).

In Figure 4 Bland-Altman plots of the inulin clearance are shown for the sampling strategies 9 (90 / 240 min) and 10 (240 min). The mean difference of the predicted and the reference inulin clearance is –0.8 and –1.2 for strategy 9 and 10, respectively. For both sampling strategies a difference of more than 10 mL/min/1.73m² was found for only one patient. The difference between the predicted inulin clearance and the reference clearance showed a larger variation at higher GFR.

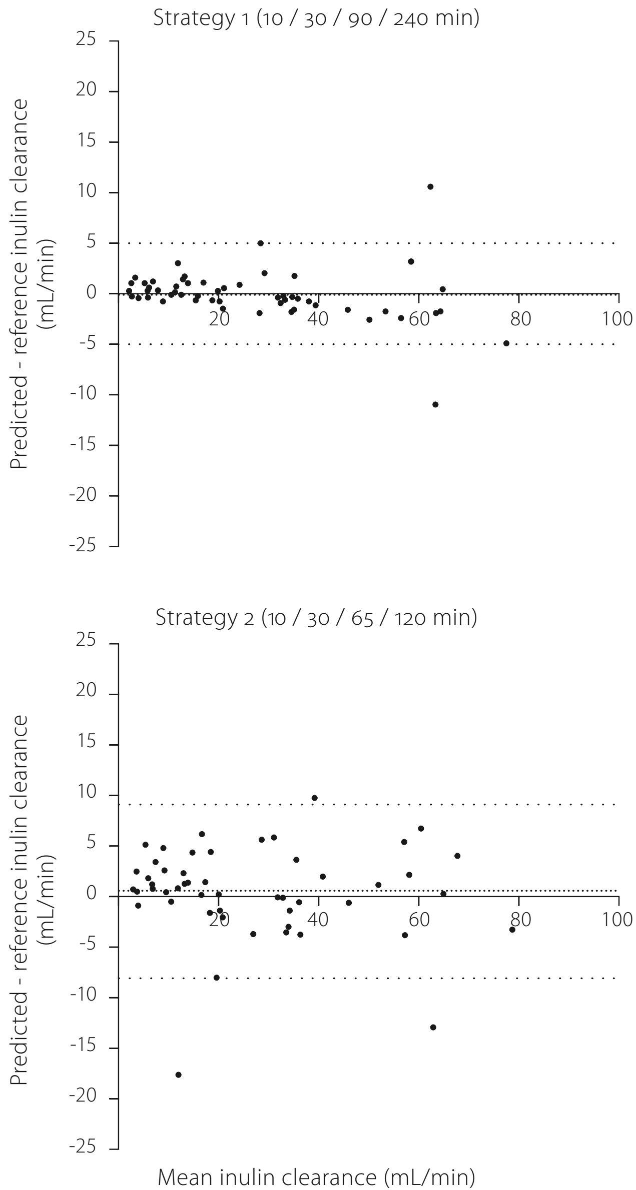


Figure 3

Bland-Altman plots of the predicted and reference inulin clearance in the validation data set for sampling strategy 1 (10 / 30 / 90 / 240 min) and 2 (10 / 30 / 65 / 120 min) (······ mean difference, · · · 2s limits).

see pages 60 - 63 for references

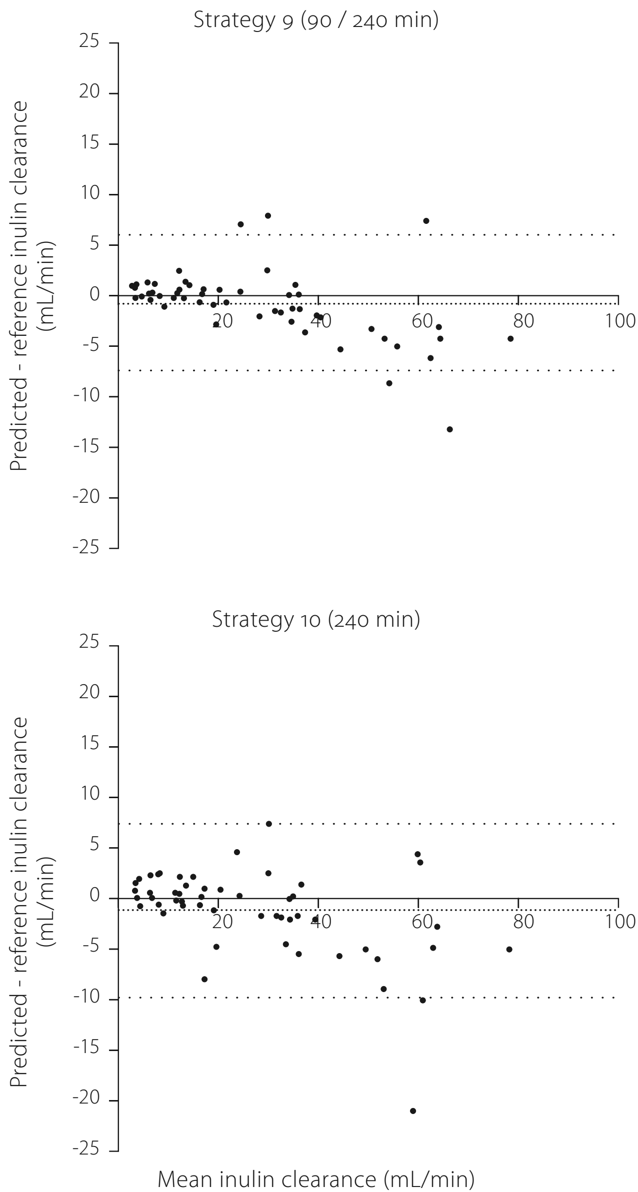


Figure 4

Bland-Altman plots of the predicted and reference inulin clearance in the validation data set for the sampling strategies 9 (90 / 240 min) and 10 (240 min) (······mean difference, · · · 2s limits).

DISCUSSION

For assessing renal function and the rate of progression of renal diseases, reliable measurement of GFR is necessary. The reference method ('gold standard') to assess GFR in children (in a research setting) is the determination of renal clearance of inulin during a continuous intravenous infusion. However, this method is complex, invasive and time consuming. Alternatively, the plasma clearance of inulin can be assessed by a single injection of inulin, which is less complex. After administration of a single dose of inulin, serial blood samples are withdrawn to construct a plasma concentration-time profile. The plasma clearance of inulin can be calculated by dividing the dose by the area under the plasma concentration-time curve. For practical and convenient application in children it is important that the total number of blood samples is minimized. The present study focused on the development of an optimal sampling strategy for the inulin single injection method in pediatric patients. It was investigated whether inulin clearance could be estimated accurately with a reduced number of blood samples.

A population pharmacokinetic model of inulin was developed based on the plasma concentration-time profiles of 100 pediatric patients in the index data set. During nonlinear mixed effect modeling the pharmacokinetic model was fitted to the data of all patients simultaneously. Typical pharmacokinetic parameters and their corresponding interindividual variability were estimated.

Inulin data were adequately described by a two-compartment model with first-order elimination from the central compartment. In literature both two- and three-compartment models have been used to describe the disposition of inulin [3, 18]. In the NONMEM analysis addition of a second peripheral compartment did not improve the fit of model to the data. This can be explained by the fact that the total sampling time in the present study was limited to 240 min. Odeh et al. described the pharmacokinetics of inulin based on a 3-compartment model. However, in that study samples were collected until 480 min after injection [18].

The covariates BSA and HUS correlated significantly with the inulin clearance and were included in the final model. They reduced the inter-individual variability from 150 to 56% and therefore explained 94% of the variability between the subjects. BSA was found as a significant covariate, which is to be expected since GFR varies with body size. For children GFR is usually expressed per 1.73m² BSA (i.e. mL/min/1.73m²) for standardization and comparison between individuals of different sizes. Patients with HUS had on average a higher clearance compared with patients with other disorders (factor 2.46). This may be explained by the fact that the patients

see pages 60 - 63 for references

with HUS in our data set were totally or partly recovered from HUS and had a (nearly) normal renal function (all patients with HUS in the validation set had a inulin clearance >100 mL/min/1.73m²). Kinowski et al. found also BSA as a significant covariate for clearance and volume of distribution of inulin in adults with diabetes or obesity [19].

The population pharmacokinetic model described the data adequately (Figure 2) and showed a low residual variability (proportional error of 7.5%). The validity of the population model was demonstrated by performing an identical analysis with the validation data set, yielding comparable results for mean pharmacokinetic parameters, variance and relationships with covariates.

Since 11 blood samples were available for each patient the Standard Two Stage method may also be used to produce population parameter estimates. Although application of this method usually produces unbiased mean estimates of parameters, random effects (variance and covariance) are likely to be overestimated in all realistic situations [20].

D-optimality theory was used for the selection of optimal sampling times. From the optimal sampling times different sampling strategies with one to four sampling times were developed and tested for the validation data set using Bayesian estimation. With Bayesian estimation the individual pharmacokinetic parameters are estimated using the derived population model and the plasma concentrations at the selected sampling times. Application of Bayesian analysis provides the advantage of estimating a pharmacokinetic parameter using only a few sampling times. Furthermore, there is no need for the sampling times to be exact. This is an advantage over other methods like multivariate linear regression techniques, which requires that the actual sample is taken at the specified, optimal sampling time [21, 22]. The Bayesian estimation method is implemented in several commercially available pharmacokinetic programs (for example NONMEM, PKS, USC-PACK, P-PHARM and MW/Pharm).

For all of the tested sampling strategies, it was found that the inulin clearance was assessed well by Bayesian estimation when at least a sample at 240 min was included. Limiting the total sampling time window from 0-240 to 0-120 min (strategy 2) resulted in a reduction of the predictive performance, indicating that the sampling time of 240 minutes after injection is critical for adequate estimation of the inulin clearance. One study in children and adults described that a total sampling time of 180 min was sufficient to estimate the inulin clearance [23]. However, in that study the predictive performance of the strategy was not tested and only a minority of patients presented a CL < 40 ml/min/1.73m². In our study the predictive performance of the sampling strategy with even one sample at 240 min (strategy 10) was acceptable for CL < 40 ml/min/1.73m² (bias 4.8 % (95% CI: -0.1 to 9.6), imprecision 14.6 % (95% CI: 0 to 20.6)). The sampling strategies 1 (10 / 30 / 90 / 240 min), 4 (10 / 30 / 240 min), 5 (10 / 90 / 240 min), 6 (30 / 90 / 240 min), and 9 (90 / 240 min)

produced accurate predictions of inulin clearance with a bias not significantly different from zero and an imprecision not exceeding 15%. The predictive performance of strategy 10 with only 1 sample taken at 240 min was acceptable as well (bias 1.0 % (95% CI: -4.0 to 5.9) and an imprecision 18.2 % (95% CI: 12.1 to 22.7)). These results are in accordance with a previously reported study on the development of a limited sampling model using Bayesian estimation in adults with diabetes or obesity, showing that with one or two samples the inulin clearance was well estimated [19]. Swinkels et al. reduced the total number of required blood samples from 11 to 6 for the determination of the inulin clearance in children [5]. Four samples were used to describe the distribution phase and 2 samples to describe the elimination phase. Since a model built in the program SAS was used for non-linear fitting it was not possible to reduce the required number of blood samples to less than 4. This applies also for the results of Florijn et al. (SIPHAR program) and Orlando et al. (GraphPad Prism) in adults [2,3]. However, with Bayesian estimation, which combines population information and individual information, it is possible to reduce the total number of required blood samples to as low as 1 [15]. In addition no optimal sampling strategies were developed in the study of Swinkels et al. and the model was not validated. Several other authors described the simplification of the inulin single injection method for the determination of the GFR in adults by using the slope clearance method instead of Bayesian estimation [24-29]. The slope clearance method is based on calculation of the AUC by the slope (rate constant) and the extrapolated inulin concentration at 0 minutes (Y- intercept). However, in nearly all those studies no optimal sampling times were determined and the simplified method was not validated.

see pages 60 - 63 for references

In conclusion, the sampling strategies of two to four sampling times and a total sampling time of 240 min result in a good prediction of inulin clearance in children with a non significant bias and a good imprecision (< 15%). The sampling strategy with two samples (90 and 240 min) seems very practical but one relies heavily on impeccable pre-analytical procedure and assay of both samples with no room for errors. Four sampling times (10 / 30 / 90 / 240 min) provide additional accuracy. Even the sampling strategy with 1 blood sample at 240 min could be used in certain cases. With the development of optimal sampling strategies for the inulin single injection method the burden of repetitive blood sampling from children to assess the GFR by the inulin single injection method can be reduced considerably. As a result, the determination of GFR based on the approach presented in this paper becomes more practical and offers more patient convenience, without compromising accuracy.

REFERENCES

1. Dalton RN, Haycock GB. Laboratory investigation. In: Barratt TM, Avner ED, Harmon WE (1999) Pediatric Nephrology, 4th edn. Lippincott Williams & Wilkins, Baltimore, pp 351-352

2. Florijn KW, Barendregt JNM, Lentjes EGWM, van Dam W, Prodjosudjadi W, van Saase JLCM et al. (1994) Glomerular filtration rate measurement by single-shot injection of inulin. Kidney Int 46:252-259

3. Orlando R, Floreani M, Padrini R, Palatini P (1998) Determination of inulin clearance by bolus intravenous injection in healthy subjects and ascitic patients: equivalence of systemic and renal clearances as glomerular filtration markers. Br J Clin Pharmacol 46:605-9

4. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM (1976) Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child 51:875-8

5. Swinkels DW, Hendriks JCM, Nauta J, de Jong MCJW (2000) GFR by single-injection inulin clearance: definition of a workable protocol for children. Ann Clin Biochem 37:60-66

6. Degenaar CP, Frenken LAM, van Hooff JP (1987) Enzymatic method for determination of inulin. Clin Chem 33:1070-1071

7. U.S. Department of Health and Human Serviced, Food and Drug Administration (1999) Guidance for Industry. Population pharmacokinetics htttp://www.fda.gov/cder/guidance/1852fnl.pdf (Accessed September 2002)

8. White DB, Walawander CA, Liu DY, Grasela TH (1992) Evaluation of hypothesis testing for comparing two populations using NONMEM analysis. J Pharmacokinet Biopharm 20: 295-313

9. Maitre PO, Buhrer M, Thomson D, Stansky DR (1991) A threestep approach combining Bayesian regression and NONMEM population analysis: application to midazolam. J Pharmacokinet Biopharm 19: 377-384

10. Mandema JW, Verotta D, Sheiner LB (1992) Building population pharmacokinetic-pharmacodynamic model. I. Models for covariate effects. J Pharmacokin Biopharm 20:511-528

11. Ette E (1998) Statistical graphics in pharmacokinetics and pharmacodynamics: a tutorial. Ann Pharmacother 32: 818-828

12. Jonsson EN, Karlsson MO (1999) Xpose- an S-PLUS based popualtion pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 58:51-64

see pages 60 - 63 for references

13. D'Argenio DZ (1981) Optimal sampling times for pharmacokinetic experiments. J Pharmacokin Biopharm 9:739-756

14. D'Argenio DZ, Schumitzky A (1997) Adapt II User's Guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software, Biomedical Simulations Resource. Release 4. University of Southern California, Los Angeles

15. Schoemaker RC, Cohen AF (1996) Estimating impossible curves using NONMEM. Br J Clin Pharmacol 42:283-90

16. Peck CC, Beal SL, Sheiner LB, Nichols AI (1984) Extended least squares nonlinear regression: a possible solution to the "choice of weights" problem in analysis of individual pharmacokinetic data. J Pharmacokinet Biopharm 12:545-58

17. Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokin Biopharm 9:503-51

18. Odeh YK, Wang Z, Ruo TI, Wang T, Frederiksen MC, Pospisil PA et al. (1993) Simultaneous analysis of inulin and 15N_2-urea kinetics in humans. Clin Pharmacol Ther 53:419-25

19. Kinowski J, Bressolle F, Rodier M, Augey V, Fabre D, Richard JL et al. (1995) A limited sampling model with Bayesian estimation to determine inulin pharmacokinetics using the population data modeling program P-PHARM. Clin Drug Invest ;9:260-269

20. Steimer, JL, Mallet A, Golmard JL, Boisvieux JF (1984) Alternative approaches to estimation of population pharmacokinetic parameters: comparison with the nonlinear mixed-effects model. Drug Metab Rev 15:265-292

21. Jodrell DI, Murray LS, Hawtof J, Graham MA, Egorin MJ (1996) A comparison of methods for limited-sampling strategy design using data from a phase I trial of the anthrapyrazole DuP-941. Cancer Chemother Pharmacol 37:356-362

22. Sorensen BT, Stromgren A, Jakobsen P, Jakobsen A (1993) A limited sampling method for estimation of carboplatin area under the curve. Cancer Chemother Pharmacol 31:324-327

23. Muller-Suur R, Goransson M, Olsen L, Backlund G, Backland L (1983) Inulin single injection clearance. Microsample technique useful in children for determination of glomerular filtration rate. Clin Physiol 3:19-27

24. Buclin T, Sechaud R, Bertschi AP, Decosterd LA, Belaz N, Appenzeller M, et al. (1998) Estimation of glomerular filtration rate by sinistrin clearance using various approaches. Ren Fail 20:267-76

25. Gretz N, Ecker-Tschirner KH, Kuhnle HF, von Dahl K, Kirschfink M, Drescher P et al. (1990) Practicability of the inulin plasma single-shot clearance. Contrib Nephrol 81:220-8

26. Gretz N, Braun C, Strauch M (1993) Determination of inulin clearance by using a single-sample method. Clin Nephrol 40:124

27. Jung K, Henke W, Schulze BD, Sydow K, Precht K, Klotzek S (1992) Practical approach for determining glomerular filtration rate by single-injection inulin clearance. Clin Chem 38:403-7

28. Prescott LF, Freestone S, McAuslane JA (1991) Reassessment of the single intravenous injection method with inulin for measurement of the glomerular filtration rate in man. Clin Sci 80:167-76

29. Schumann L, Wustenberg PW, Hortian B, Kuhnle HF (1993) Determination of glomerular filtration rate (GFR) on two consecutive days using inulin in a single-sample plasma clearance method. Clin Nephrol 39:65-9

see pages 60 - 63 for references

CHAPTER 3

DETERMINATION OF INULIN CLEARANCE BY SINGLE INJECTION OR INFUSION IN CHILDREN

L.K. van Rossum[1], K. Cransberg[2], Y.B. de Rijke[3], R. Zietse[4],
J. Lindemans[3], A.G. Vulto[1]

[1] Department of Hospital Pharmacy,
[2] Department of Pediatric Nephrology,
[3] Department of Clinical Chemistry,
[4] Department of Internal Medicine,
Erasmus MC, Rotterdam, The Netherlands

Pediatric Nephrology, 2005;20:777-781

see page 75 for references

ABSTRACT

The reference method to determine the glomerular filtration rate (GFR) in children is the urinary clearance of inulin during a continuous intravenous infusion. Alternatively, the plasma clearance of inulin can be determined, which does not require urine collection. The aim of this study was to compare the inulin plasma clearance in pediatric patients determined by two methods: the single injection and the continuous infusion method.

The inulin plasma clearance was determined in 24 patients by both methods. In the single injection method 5000 mg/m² of inulin (Inutest®)was administered as bolus injection and blood samples were drawn at 10, 30, 90 and 240 minutes after administration. For the continuous infusion method inulin was started overnight and blood samples were collected the next day.

The inulin plasma clearance determined by the single injection method was on average 9.7 mL/min/1.73m² higher than the clearance determined with the continuous infusion method (95% CI: 5.3; 14.2). The difference between both methods was smaller at lower GFRs.

The difference in results generated by the two methods in children is small and is considered acceptable in clinical practice. For practical reasons, the single injection method with minimum sampling is preferred.

INTRODUCTION

Glomerular Filtration Rate (GFR) is considered the most fundamental parameter in the evaluation of renal function in suspected renal diseases and in monitoring renal function during treatment. The ideal marker for GFR determination would be physiologically inert, freely filtered in the glomerulus, neither secreted nor reabsorbed by the renal tubule, and not synthesized or metabolized by the kidney. Inulin, an exogenous polysaccharide, has all of these properties. Consequently, the urinary clearance of inulin during continuous intravenous infusion of inulin is regarded as the gold standard for determining GFR in children [1]. However, this method requires an intravenous infusion of inulin and timed urine collections over a period of several hours, which is difficult and needs bladder catheterization, especially in smaller children. As a result, alternative methods, without collection of urine, have been developed.

The plasma clearance of inulin can be measured after either a continuous intravenous infusion or a single bolus injection. The continuous infusion method is based on the concept that when the plasma concentration of the marker is constant (steady state concentration) and the volume of distribution is saturated with the marker (state of equilibrium), the rate of excretion equals the rate of infusion [1, 2]. The time to reach a steady state concentration is critical, since GFR is overestimated if a steady state concentration has not been reached. An equilibration period of one to three hours is common but there are data indicating that a period of more than 12 hours may be required for a complete equilibrium of inulin in the extracellular volume [3, 4, 5]. In general the steady state concentration of a drug (90% of the steady state) is reached in 3.3 half-lives [6]. Therefore in the case of decreased renal function the time to achieve steady state is delayed. However, a loading dose can be given to reach the steady state concentration more rapidly.

In the Sophia Children's Hospital, the inulin plasma clearance with continuous infusion has been used as the standard method to determine GFR for many years. With this method, hospitalization is necessary for the duration of the inulin infusion and while three capillary blood samples are drawn. This makes it both time-consuming and inconvenient.

The single injection method can be performed in daycare, with only one venous puncture for the administration of inulin and for the collection of blood samples. A bolus of inulin is administered intravenously and blood samples are collected up to 240 min after injection. The inulin concentrations measured are used to construct

see page 75 for references

a plasma concentration vs time decay curve [1]. For an accurate description of the curve 10-12 blood samples are usually required. It is stressful for children to draw a lot of blood samples and therefore limited and optimal sampling strategies were introduced [7, 8]. As a result the inulin single injection method is more convenient for patients and nurses.
In adults good agreement has been reported between the plasma clearance of inulin determined by the single injection method and the continuous infusion method [9, 10]. No similar comparison has been made in children.
The aim of this study was to compare the plasma clearance of inulin determined by the single injection method and the continuous infusion method in pediatric patients.

METHODS

Patients

The study was approved by the Medical Ethics Committee of the hospital and informed consent was obtained from patients or their parents. Twenty-four pediatric patients with stable renal function were included between March 2000 and November 2002. All the patients were hospitalized at the Sophia Children's Hospital of Rotterdam for various reasons. Patient characteristics are summarized in Table I. Two patients were included twice: one patient showed a decrease in GFR (from 20 to 12 mL/min/1.73m²) and the other patient received a renal transplant and had GFRs of 14 and 95 mL/min/1.73m², before and after transplantation, respectively.

Study design

In all patients a single cannula was inserted into an antecubital vein. The single injection method started in the morning at 9.00 h. From midnight before the diet was restricted: no fruit, lemonade, or caffeinated beverages were allowed. Inulin (polyfructosan, Inutest®, Fresenius Pharma, Graz, Austria) was infused in a dose of 5000 mg/1.73 m² body surface area (maximum dose 5000 mg), at a constant rate over 30 seconds. The precise dose was determined by weighing the syringe before and after injection on a highly accurate balance. After administration of inulin the cannula was flushed with 20 ml of 0.9% saline. Blood samples (heparin samples) of 1 ml were taken at 0, 10, 30, 90 and 240 minutes after injection. Between sampling the cannula was kept open with a heparin lock. To prevent dilution of a blood sample with heparin the first 2.5 mL of blood was drawn prior to sampling and, if possible, re-injected after sampling.
At the end of the single injection method the continuous infusion method was started. The infusion rate was calculated as follows [9]:

$$R = \frac{2 \times BH \times BSA}{Pcr}$$

in which R is the infusion rate of inulin 25% solution (mL/h), BH is the body height (cm), BSA is body surface area (m²) and Pcr is the plasma concentration of creatinine (µmol/L).

Table I. Patient characteristics[1]

Patients	24
male / female	11 / 13
Age (years)	10.3 (2.4 - 17.9)
Weight (kg)	24 (13 – 48)
Height (cm)	128 (88 – 165)
BSA (m^2)	0.93 (0.55 – 1.50)
BMI (kg/m^2)	16 (13 – 20)
Disorders	
renal transplantation	9
chronic renal failure	5
acute renal failure	1
other renal disorders	4
heart transplantation	1
urological disorders	1
other disorders	3
GFR ($mL/min/1.73m^2$)[2]	42.0 (10.6 – 125.9)

[1]Data are presented as median (range)

[2]GFR: plasma clearance of inulin determined by the continuous infusion method

BSA: body surface area; BMI: body mass index

Table II. Population pharmacokinetic parameters of inulin

		Mean	Coefficient of variation (%)
CL	($L/h/1.85m^2$)	2.02	61
V_1	(L/kgLBMc)	0.0655	57
Q	($L/h/1.85m^2$)	10.7	21
V_2	(L/kgLBMc)	0.119	26

CL: plasma clearance of inulin; V_1: volume of distribution of the central compartment; Q: intercompartmental clearance; V_2: volume of distribution of the peripheral compartment; LBMc: lean body mass corrected for fat distribution

see page 75 for references

During the first two hours the infusion rate was doubled to saturate the compartments faster. The infusion was continued overnight. Capillary samples were taken the next day at 11.00, 12.00 and 13.00 h and collected in heparin microtainers.

Biochemical assay

In the laboratory the blood samples were centrifuged and serum samples were stored at -20 °C.
To determine the inulin concentration, frozen samples were thawed completely and 150 µL was deproteinized by perchloric acid (1:1). Samples were then centrifuged for 10 min at 10.000 rpm (9500 g). The supernatant was neutralized with 5 M KOH, and centrifuged for another 10 min. Thereafter, concentrations of inulin were measured in the supernatant (0.1 mL) in duplicate by an automated enzymatic method [11, 12]. Inulinase hydrolyses inulin to fructose and sorbitol dehydrogenase converts fructose to sorbitol with the consumption of NADH, which is detected spectrofotometrically on a Hitachi 912 (Roche Diagnostics). The analytical coefficient of variation for this method was 6.9% at 136 mg/L and 1.9% at 725 mg/L. The assay was linear up to 2100 mg/L.

Pharmacokinetic analysis

For the single injection method the inulin plasma clearance was obtained by fitting the plasma concentration-time decay curve of inulin to a two-compartment model by Bayesian analysis using the pharmacokinetic software program MW/Pharm version 3.50 (Mediware, Groningen, the Netherlands).
The basis of Bayesian analysis is that for estimation of the individual pharmacokinetic parameters information from the population pharmacokinetic parameters is combined with information derived from the actual individual concentrations of the samples. A weighted combination of individual and population information where the weighting depends on how much information the individual itself supplies, is applied. The population pharmacokinetic parameters of inulin are presented in Table II. If a large number of accurate blood samples is available the Bayesian estimation will largely be determined by the concentration of those samples alone. If, in contrast, only 1 blood sample is available the Bayesian estimation will largely be determined by the information obtained from the population pharmacokinetic parameters. More information from the population pharmacokinetic parameters is added to the actual concentrations with decreasing number of samples.
For the continuous infusion method GFR was calculated as follows:

$$GFR\ (mL/\text{min}) = \frac{R \times 250}{Pi \times 60}$$

in which R is the infusion rate (mL/h) and Pi the mean plasma concentration of inulin from the capillary samples (mg/mL).
GFR was corrected for body surface area (mL/min/1.73m²).

Statistical analysis

Results were presented as median and range. The correlation coefficient (R^2) was used to describe the strength of the relation between the inulin single injection method and the continuous infusion method. Agreement between the two methods was evaluated as described by Bland and Altman [13]. The limits of agreement represent the range around the mean difference between both methods in which 95% of the values will be found (mean difference ± 1.96 x SD). The 95% confidence intervals of the mean difference were calculated by the mean difference ± t x standard error of the mean difference.
The statistical analysis was made by using the GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA).

RESULTS

After overnight infusion of inulin a steady state concentration was reached for the continuous infusion method in all patients (difference between the samples < 15% except in 1 patient). The steady state concentration of inulin varied from 312 to 1006 mg/L. In 3 patients one of the capillary blood samples was not available due to a (pre-) analytical error.
Table III presents the individual plasma clearance of inulin for the single injection method and the continuous infusion method. GFR found by the single injection method varied from 13.6 to 150.9 mL/min/1.73m². The relationship between the plasma clearance of inulin determined by the single injection method with 4 blood samples (10 / 30 / 90 / 240 min) and the inulin plasma clearance based on the continuous infusion method is shown in Figure 1. The coefficient of correlation was 0.92. The inulin plasma clearance determined by the single injection method was higher than the inulin plasma clearance based on the continuous infusion method at all levels of GFR, except in 2 patients. The mean inulin plasma clearance for the single injection method was 61.6 mL/min/1.73m², while a mean plasma clearance of 51.9 mL/min/1.73m² was found for the continuous infusion method. The mean difference in inulin plasma clearance between the single injection method and the continuous infusion method was 9.7 mL/min/1.73m² (Figure 2).
The 95% confidence interval for the mean difference was 5.3 - 14.2 mL/min/1.73m², which shows that the mean difference was statistically significant. Limits of agreement (mean difference ± 1.96 SD) were - 10.9 and 30.4 mL/min/1.73m².
The difference between both methods was smaller at lower GFRs.

see page 75 for references

Table III. Individual plasma clearance of inulin (mL/min/1.73m²) determined by the single injection method and the continuous infusion method (n=24)

	Inulin clearance determined by	
Patient no.	single injection (mL/min/1.73m²)	continuous infusion (mL/min/1.73m²)
1	150.9	125.6
2	38.3	27.7
3	66.6	54.6
4	21.7	14.2
5	27.3	21.0
6	53.9	39.8
7	48.8	42.0
8	34.4	26.9
9	56.9	41.9
10	22.3	16.3
11	123.6	125.9
12	101.8	124.7
13	116.4	101.2
14	18.9	12.9
15	47.7	47.1
16	56.6	43.3
17	48.4	43.4
18	95.9	83.4
19	13.6	10.6
20	55.5	40.1
21	105.9	80.8
22	49.8	37.6
23	99.0	66.6
24	23.9	16.8

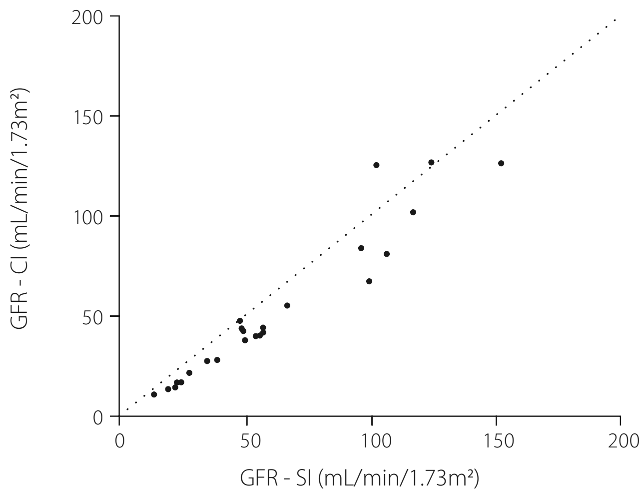


Figure 1

Correlation between the plasma clearance of inulin determined by the single injection method (GFR-SI) and by the continuous infusion method (GFR-CI) (······ line of identity; Y = -5.384 + 0.9293 X).

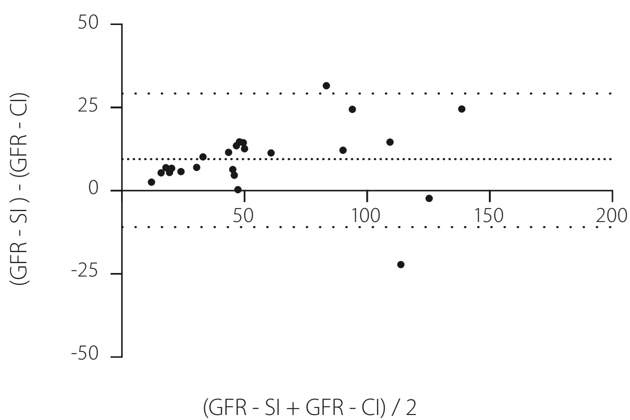


Figure 2

Bland-Altman plot of the inulin plasma clearance determined by the single injection method (GFR-SI) and the continuous infusion method (GFR-CI) (······ mean difference, ··· limits of agreement).

see page 75 for references

For GFRs < 50 mL/min/1.73m² a mean difference of 8.5 mL/min/1.73m² (95% CI: 6.2; 10.9; limits of agreement: –0.11; 17.2), while for GFRs > 50 mL/min/1.73m² a mean difference of 12.2 mL/min/1.73m² (95% CI: -3.5; 27.0; limits of agreement: –22.6; 46.9) was observed.

DISCUSSION

The inulin plasma clearance determined by the single injection method correlated well with the continuous infusion method. The single injection method resulted in an inulin plasma clearance, that was, on average, 9.7 mL/min/1.73m² higher than the clearance determined with the continuous infusion method. This mean difference was statistically significant. The mean difference was smaller for GFRs < 50 mL/min/1.73m², which are the GFRs of clinical interest. It is important to realize that bias in the continuous infusion method could also account for this difference. Theoretically it is even possible that the single injection method is a more accurate representation of the urinary clearance of inulin than the continuous infusion method. Unfortunately, the trial was not designed to investigate this point.

The findings are in accordance with the results of Florijn et al., who compared the plasma clearance of inulin determined by the single injection method with the continuous infusion method in 14 adult patients with autosomal-dominant polycystic kidney disease with a GFR range of 27 - 137 mL/min/1.73m² [9]. In that study a correlation coefficient (r) of 0.95 was found and an overestimation was observed for the inulin single injection method (mean difference between both methods 7 mL/min/1.73m²).

The difference in results between the single injection and the continuous infusion method cannot be explained by the use of venous versus capillary blood samples. Müller-Suur et al. compared the inulin plasma clearance with single injection based on venous and capillary blood samples in 21 children [14]. Good agreement between the two methods was found (r=0.94). Bäcklund et al. studied the same relationship in 31 children and adults. In that study a correlation coefficient of 0.89 was observed [15]. We compared the inulin concentration in venous and capillary blood samples in 13 children (data not shown). In general the inulin concentration corresponded well between the blood samples (mean difference 12 mg/L, not statistically significant). A circadian rhythm for GFR has been described [16]. Since the blood samples were taken at similar times for both methods, it is unlikely that this explains the difference.

Determination of the urinary clearance of inulin during continuous intravenous infusion of inulin is regarded as the gold standard to determine GFR in children. Since no comparison was made, it is not clear which method will agree better with the urinary clearance of inulin. Determination of the urinary clearance of inulin requires an intravenous infusion of inulin and timed urine collections over a period of several hours, which is difficult and needs bladder

catheterization, especially in smaller children. Urine samples are not required to determine the plasma clearance of inulin.
The single injection method has several advantages over the continuous infusion method in determining the plasma clearance of inulin. The former method takes 4 hours while the continuous infusion method generally requires an overnight stay since a steady state concentration has to be reached [3]. It is important for patients that the total number of blood samples required is minimal. The number of blood required was nearly similar in the two methods (4 for the single injection method and 3 for the continuous infusion method). However, the continuous infusion method required capillary blood samples since it was not possible to draw blood from the cannula while the inulin was being infused.

In conclusion, determination of the inulin plasma clearance in children with the single injection method resulted in a small but not clinically relevant overestimation of the inulin plasma clearance compared with the continuous infusion method. The discrepancy between the results was smaller at GFRs below 50 mL/min/1.73m². The practical advantages (no overnight stay and less cumbersome) make the inulin single injection method with minimal blood sampling preferable for the determination of the plasma clearance of inulin in children.

see page 75 for references

REFERENCES

1. Barratt TM, Avner ED, Harmon WE (1999) Pediatric Nephrology, 4th edn. Lippincott Williams & Wilkins, Baltimore, pp 351-352

2. Earle DP, Berliner RW (1946) A simplified clinical procedure for measurement of glomerular filtration rate and renal plasma flow. Proc Soc Exp Biol Med 62: 262-264

3. Hellerstein S, Berenbom M, Alon U, Warady BA (1993) The urinary clearance and infusion clearance of inulin are similar, but not identical. Kidney Int 44:1058-1061

4. Coulthard MG (1983) Comparison of methods of measuring renal function in preterm babies using inulin. J Pediatr 102:923-930

5. Van der Heijden AJ, Grose WF, Ambagtsheer JJ, Provoost AP, Wolff ED, Sauer PJ (1988) Glomerular filtration rate in the preterm infant: the relation to gestational and postnatal age. Eur J Pediatr 148:24-28

6. Rowland M, Tozer T (1994) Clinical pharmacokinetics, concepts and application, 3rd edn. Williams & Wilkins, USA, pp 70-71

7. Swinkels DW, Hendriks JCM, Nauta J, de Jong MCJW (2000) GFR by single-injection inulin clearance: definition of a workable protocol for children. Ann Clin Biochem 37:60-66

8. Van Rossum LK, Mathot RAA, Cransberg K, Vulto AG (2003) Optimal sampling strategies to assess inulin clearance in children by the inulin single injection method. Clin Chem 49:1170-1179

9. Florijn KW, Barendregt JNM, Lentjes EGWM, van Dam W, Prodjosudjadi W, van Saase JLCM et al. (1994) Glomerular filtration rate measurement by single-shot injection of inulin. Kidney Int 46:252-259

10. Orlando R, Floreani M, Padrini R, Palatini P (1998) Determination of inulin clearance by bolus intravenous injection in healthy subjects and ascitic patients: equivalence of systemic and renal clearances as glomerular filtration markers. Br J Clin Pharmacol 46:605-609

11. Summerfield AL, Hortin GL, Smith CH, Wilhite TR, Landt M (1993) Automated enzymatic analysis of inulin. Clin Chem 39:2333-2337

12. Wong EC, Wilhite TR, Miller R, Smith CH, Landt M (1994) Less expensive enzymatic analysis for inulin with a kinetic assay. Clin Chem 40:1788-1789

13. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307-310

14. Muller-Suur R, Goransson M, Olsen L, Backlund G, Backlund L (1983) Inulin single injection clearance. Microsample technique useful in children for determination of glomerular filtration rate. Clin Physiol 3:19-27

15. Backlund L, Goransson M, Muller-Suur R, Olsen L (1983) Evaluation of glomerular filtration rate in infants. Acta Paediatr Scand Suppl 305:77-8

16. Koopman MG, Koomen GC, Krediet RT, de Moor EA, Hoek FJ, Arisz L (1989) Circadian rhythm of glomerular filtration rate in normal individuals. Clin Sci 77:105-11

CHAPTER 4

BEPALING VAN DE GLOMERULAIRE FILTRATIESNELHEID BIJ KINDEREN DOOR MIDDEL VAN DE INULINE SINGLE INJECTIE METHODE

L.K. van Rossum[1], K. Cransberg[2], R.A.A. Mathot[1], A.G. Vulto[1]

[1] Afdeling Ziekenhuisapotheek,

[2] Afdeling Kindernefrologie,

Erasmus MC, Rotterdam, Nederland

Tijdschrift voor Kindergeneeskunde: 2005;73:170-175

see pages 88 - 89 for references

ABSTRACT

Renal function can be assessed by determination of the glomerular filtration rate (GFR). The standard method to determine GFR in children is the urinary clearance of inulin during a continuous intravenous infusion. However, this method is complex and invasive. Alternatively, the plasma clearance of inulin can be determined by single injection of inulin. The aim of this study was to validate the single injection method with a limited number of blood samples in pediatric patients.
Determination of the inulin plasma clearance by single injection was performed in 48 patients. A single injection of inulin (Inutest®, 5000 mg/m²) was administered and blood samples were drawn at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180 and 240 minutes after administration. The inulin plasma clearance based on 4 blood samples (10, 30, 90 and 240 min) was compared with the inulin plasma clearance based on all blood samples (reference clearance).
In patients with GFR < 75 mL/min/1.73m² the mean difference between the inulin plasma clearance based on 4 blood samples and the reference clearance was not statistically significant. For GFR > 75 mL/min/1.73m² a small bias was observed (mean difference: -5.92 mL/min/1.73m²; 95% confidence interval: -10.2; -1.68 mL/min/1.73m²).
In patients with decreased renal function the inulin plasma clearance determined by the single injection method with 4 blood samples agreed well with the inulin plasma clearance based on 11 blood samples. As a consequence, it is a simple and convenient method to determine the renal function in pediatric patients.

INLEIDING

De glomerulaire filtratiesnelheid (GFR) is de belangrijkste parameter om de nierfunctie te volgen bij nierziekte en tijdens behandeling met geneesmiddelen. De ideale marker om de nierfunctie te bepalen is fysiologisch inert, wordt vrij gefiltreerd in de glomerulus, kent geen tubulaire secretie en wordt niet gereabsorbeerd, gesynthetiseerd of gemetaboliseerd in de nieren. Inuline, een exogeen polysaccharide, wordt beschouwd als de 'gouden marker' omdat het alle eigenschappen van een ideale marker heeft [1]. Zowel de urine klaring als de plasma klaring van inuline kan worden bepaald. Bij het bepalen van de plasma klaring van inuline wordt het verdwijnen van inuline uit het plasma in de loop van de tijd gevolgd. Inuline wordt via continue infusie (inuline continue infusiemethode) of eenmalige injectie (inuline single injectie methode) toegediend. De inuline single injectie methode, waarbij inuline als bolus injectie intraveneus wordt toegediend en op een aantal tijdstippen bloedmonsters worden afgenomen voor het opstellen van de plasma verdwijningscurve, is in een aantal opzichten praktischer. De totale periode voor het bepalen van de inuline plasma klaring kan beperkt blijven tot 4 uur en er hoeft slechts eenmaal geprikt te worden voor het inbrengen van een afname-canule. Voor het nauwkeurig beschrijven van de plasma verdwijningscurve was tot voor kort een groot aantal bloedmonsters nodig (10-12). Door het opstellen van gelimiteerde en optimale bemonsteringsschema's voor de inuline single injectie methode is het aantal af te nemen bloedmonsters sterk gereduceerd, waardoor de methode patiëntvriendelijker is geworden [2, 3]. In dit artikel wordt de praktische uitvoering van de inuline single injectie methode met een beperkt aantal bloedmonsters voor het bepalen van de inuline plasma klaring bij kinderen en de validatie ervan beschreven.

see pages 88 - 89 for references

METHODEN

Patiënten

Het onderzoek vond plaats op de afdeling Thuisdialyse van het Erasmus MC-Sophia in Rotterdam. Tussen april 2000 en augustus 2002 ondergingen 48 kinderen een test voor het bepalen van de inuline plasma klaring. Deze test werd 's ochtends gestart. Inuline (polyfructosaan, Inutest®, Fresenius Pharma, Graz, Oostenrijk) werd via een canule in een antecubitale vene in een dosis van 5000 mg/1.73 m² lichaamsoppervlak (maximale dosis 5000 mg) met een constante snelheid in 30 seconden geïnjecteerd. De precieze dosis inuline werd bepaald door de spuit voor en na het geven van inuline te wegen. Na toediening van inuline werd de canule doorgespoeld met 20 ml natriumchloride 0.9%. Bloedmonsters (1 ml) werden afgenomen op de tijdstippen 0, 10, 20, 30, 45, 60, 90, 120, 150, 180 en 240 min na toedienen van inuline [2]. Na het afnemen van elk monster werd 1 mL heparine (4 IE/mL) toegediend. Om verdunning van het af te nemen bloedmonster te voorkomen werd de eerste 2.5 mL bloed terzijde gelegd en indien mogelijk na het afnemen van het bloedmonster teruggegeven.

Biochemische analyse

De bloedmonsters werden bij binnenkomst gecentrifugeerd en serum of heparine plasma monsters werden opgeslagen bij -20 °C. Voor het bepalen van de inuline concentratie werden de monsters ontdooid en onteiwit met perchloorzuur. De inuline concentratie werd gemeten met een geautomatiseerde enzymatische methode (Hitachi 912, Roche Diagnostics) [4, 5]. De variatiecoëfficiënt voor deze methode bedroeg 6.9% bij een inuline concentratie van 136 mg/L en 1.9% bij 725 mg/L. De methode was lineair tot een concentratie van 2100 mg/L.

Farmacokinetische analyse

De farmacokinetiek van inuline werd beschreven met behulp van een 2-compartiment model. Individuele curves gebaseerd op alle afnametijdstippen werden gefit met behulp van een gemodifideerde kleinste kwadraten methode en de individuele inuline klaring werd hieruit geschat [6]. In het geval van 4 afnametijdstippen werd een schatting van de inuline klaring verkregen door Bayesiaanse analyse zoals geïmplementeerd in het farmacokinetiek programma MW/Pharm versie 3.50 (Mediware, Groningen). MW/Pharm is een farmacokinetiek programma dat in bijna elke ziekenhuisapotheek beschikbaar is. Bij Bayesiaanse analyse werden de concentraties op de 4 tijdstippen 10, 30, 90 en 240 minuten gecombineerd met de populatie farmacokinetische gegevens van inuline (Tabel I). Door combinatie van individuele en populatie gegevens kan voor een individuele patiënt met minder afnametijdstippen de inuline klaring worden geschat. De afnametijdstippen 10, 30, 90 en 240 minuten na toediening zijn afgeleid van een optimaal bemonsteringsschema voor inuline [3].

Tabel I. Populatie farmacokinetische parameters voor inuline

		Gemiddelde	Variatiecoëfficiënt (%)
CL	(L/u/1.85m²)	2.02	61
V_1	(L/kgLBMc)	0.0655	57
Q	(L/u/1.85m²)	10.7	21
V_2	(L/kgLBMc)	0.119	26

Residuale fout: 7.54%

CL: inuline plasma klaring; V_1: verdelingsvolume centraal compartiment; Q: intercompartimentele klaring; V_2: verdelingsvolume perifeer compartiment; LBMc: lean body mass gecorrigeerd voor vetverdeling

see pages 88 - 89 for references

Statistische analyse

De overeenkomst tussen de inuline plasma klaring verkregen op grond van de 4 afnametijdstippen en alle 11 afnametijdstippen (referentie inuline klaring) werd beschreven aan de hand van een Bland-Altman grafiek [7]. Het verschil in inuline klaring werd uitgezet tegen de gemiddelde inuline klaring ((inuline klaring gebaseerd op 4 afnametijdstippen + referentie inuline klaring) / 2). De limits of agreement in de Bland-Altman grafiek geven het bereik rondom het gemiddelde verschil in inuline klaring aan, waarbinnen 95% van alle waarden ligt (gemiddeld verschil ± 1.96 x SD). Het 95% betrouwbaarheidsinterval van het gemiddelde verschil in inuline klaring werd berekend door middel van het gemiddelde verschil ± t x standard error, waarbij t het getal is dat hoort bij een verdeling van n-1 vrijheidsgraden.

RESULTATEN

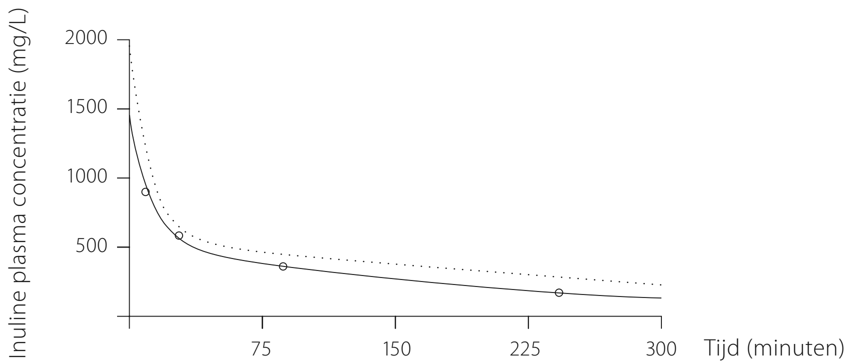
Bij 48 patiënten (Tabel II), in leeftijd variërend van 5 tot 18 jaar, werd de inuline plasma klaring bepaald. Bij het merendeel van de patiënten werd de test als routine uitgevoerd om de nierfunctie na transplantatie te volgen. Bij 13 patiënten werd de inuline

Tabel II. Patiëntkarakteristieken[1]

Patiënten	48
mannelijk / vrouwelijk	34 / 14
Leeftijd (jaren)	13 (5-18)
Gewicht (kg)	47 (20-82)
Lengte (cm)	152 (106-186)
Lichaamsoppervlakte (m^2)	1.44 (0.75-1.98)
Kreatinine concentratie (µmol/L)	89 (35-434)
Plasma klaring van inuline (mL/min/1.73m^2)	71 (7-158)
Ziektebeeld	
Niertransplantatie	38
Overig	10

[1]Data worden weergegeven als mediaan (bereik)

klaring in de aangegeven periode twee keer bepaald. De inuline plasma klaring varieerde van 7 tot 158 mL/min/1.73 m² (mediaan 71 mL/min/1.73m²). In Figuur 1 is een voorbeeld van een verdwijningscurve van inuline uit het plasma weergegeven. De Bland-Altman grafiek voor de inuline klaring op basis van 4 en 11 afnametijdstippen is in Figuur 2 weergegeven. Voor een GFR kleiner dan 75 mL/min/1.73m² werd een gemiddeld verschil van –0.60 mL/min/1.73m² met een 95% betrouwbaarheidsinterval van –1.51 tot 0.35 gevonden (n=29). Een gemiddeld verschil van –5.92 mL/min/1.73m² (95% betrouwbaarheidsinterval –10.2; -1.68) werd waargenomen bij een GFR groter dan 75 mL/min/1.73m² (n=19).



Figuur 1

Inuline verdwijningscurve uit het plasma (patiënt met BSA: 0.98 m², dosis Inutest: 3155 mg). De bovenste curve (···) geeft de curve aan op grond van de populatie farmacokinetische gegevens van inuline (inuline klaring: 31.5 mL/min/1.73m²); de onderste curve (—) is een individuele curve opgesteld met Bayesiaanse analyse, waarbij de inuline plasma concentraties op de tijdstippen 10, 30, 90 en 240 minuten (O) gecombineerd zijn met het populatiemodel (inuline klaring: 50.9 mL/min/1.73m²).

see pages 88 - 89 for references

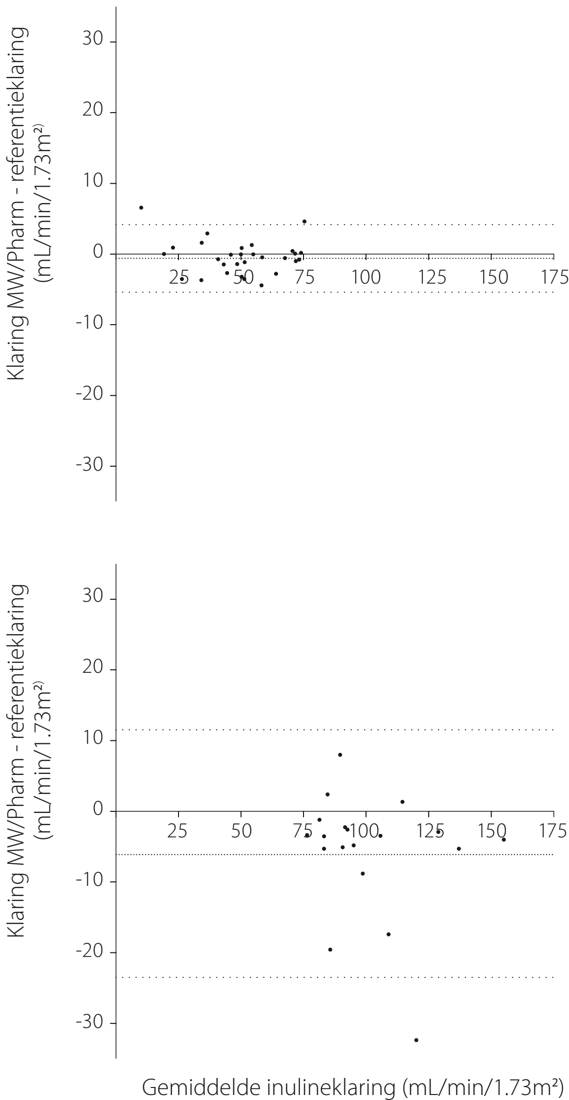


Figure 2

Bland-Altman grafiek voor de inuline plasma klaring gebaseerd op 4 afnametijdstippen (klaring MW/Pharm) en 11 afnametijdstippen (referentieklaring) voor GFR < 75 mL/min/1.73m² (bovenste figuur) en GFR > 75 mL/min/1.73m² (onderste figuur) (...... gemiddeld verschil, · · · limits of agreement).

DISCUSSIE

Voor het meten van de GFR kunnen verschillende markers worden gebruikt. De ideale marker om de nierfunctie te bepalen is fysiologisch inert, wordt vrij gefiltreerd in de glomerulus, kent geen tubulaire secretie en wordt niet gereabsorbeerd, gesynthetiseerd of gemetaboliseerd in de nieren. In het geval van een endogene marker is een constante productie snelheid eveneens een vereiste. Markers voor de GFR kunnen worden onderverdeeld in exogene en endogene markers. Tot de exogene markers behoren inuline, radio-isotopen (^{125}I-iothalamaat, ^{51}Cr-EDTA en ^{99m}Tc-DTPA) en iohexol. Kreatinine en cystatine C zijn endogene markers. Inuline, een polysaccharide, heeft alle eigenschappen van een ideale marker en wordt gezien als de 'gouden standaard'. Het gebruik van radio-isotopen als marker voor GFR bij kinderen is in Nederland beperkt vanwege veiligheidsaspecten (blootstelling aan straling). Iothalamaat kan ook in niet gelabelde vorm worden gebruikt, maar in de literatuur is tubulaire secretie van iothalamaat beschreven, waardoor het geen ideale marker voor de GFR is [8]. Iohexol is een laag osmolair non-ionogeen contrastmedium (molecuulgewicht 821 Da). Ervaring met iohexol als marker voor GFR bij kinderen is beperkt. Daarom wordt de voorkeur gegeven aan inuline als exogene marker voor het bepalen van de GFR bij kinderen.

De klassieke methode voor het bepalen van de GFR bestaat uit het toedienen van inuline via continue intraveneuze infusie en het gedurende een aantal uren verzamelen van urinemonsters. Met deze methode wordt het verschijnen van inuline in de urine gevolgd. Aangezien het lastig is om bij jonge kinderen nauwkeurig urine te verzamelen, kan als alternatief de plasma klaring van inuline worden bepaald. Het verdwijnen van inuline uit het plasma in de loop van de tijd wordt hierbij gevolgd. Bij deze methode worden geen urinemonsters verzameld. Inuline wordt via continue infusie (inuline continue infusiemethode) of eenmalige injectie (inuline single injectie methode) toegediend. Bij de continue infusiemethode wordt er vanuit gegaan dat de snelheid van uitscheiding gelijk is aan de infuussnelheid vanaf het moment dat de plasma concentratie van inuline constant is [1]. De tijd om tot een constante inuline plasma concentratie te komen is kritisch en varieert van 1 tot 12 uur [9]. De inuline single injectie methode, waarbij inuline als bolus injectie intraveneus wordt toegediend en op een aantal tijdstippen bloedmonsters worden afgenomen voor het opstellen van de plasma verdwijningscurve, is in een aantal opzichten praktischer. De totale periode voor het bepalen van de inuline plasma klaring kan beperkt blijven tot 4 uur en er hoeft

see pages 88 - 89 for references

slechts eenmaal geprikt te worden voor het inbrengen van een afname-canule. Voor het nauwkeurig beschrijven van de plasma verdwijningscurve was tot voor kort een groot aantal bloedmonsters nodig (10-12). Door het opstellen van gelimiteerde en optimale bemonsteringsschema's voor de inuline single injectie methode is het aantal af te nemen bloedmonsters sterk gereduceerd, waardoor de methode patiëntvriendelijker is geworden [2, 3]. In het Erasmus MC-Sophia is bij 24 kinderen de inuline single injectie methode vergeleken met de continue infusiemethode, wat tot dan toe de gebruikelijke methode was voor het bepalen van de GFR. De inuline plasma klaring bepaald met de single injectie methode was gemiddeld iets hoger (9.7 mL/min/1.73m²), waarbij het verschil tussen de methoden kleiner was bij een slechtere nierfunctie (GFR < 50 mL/min/ 1.73m²) [10].

Voor de dagelijkse praktijk is het bepalen van de urine klaring of de plasma klaring van inuline ongeschikt omdat het relatief veel tijd kost. In de kliniek wordt daarom in het algemeen alleen gebruik gemaakt van endogene markers (kreatinine of cystatine C). Kreatinine, een afbraakproduct van spieren, is geen ideale marker omdat de kreatinine plasma concentratie afhankelijk is van de spiermassa van de patient. Een andere reden waarom kreatinine geen geschikte parameter is voor het volgen van de nierfunctie is dat het niet alleen glomerulair gefiltreerd wordt maar ook via tubulaire secretie wordt uitgescheiden. De mate van tubulaire secretie van kreatinine is niet constant: de uitscheiding van kreatinine via tubulaire secretie ten opzichte van de totale kreatinine uitscheiding neemt toe bij een slechtere nierfunctie. Dit leidt in het geval van een slechte nierfunctie tot overschatting van de GFR [8]. Cystatine C, eveneens een endogene marker, is een eiwit (molecuulgewicht 13.3 kDa), dat het enzym cysteïne proteïnase remt. Het is een relatief nieuwe marker voor de GFR. Het wordt met een constante snelheid aangemaakt in alle kernhoudende cellen en wordt vrij gefiltreerd in de glomerulus. Door tubulaire epitheelcellen wordt het vervolgens teruggeresorbeerd en gekataboliseerd [11, 12]. Uit verschillende onderzoeken blijkt cystatine C geen betere GFR marker te zijn in vergelijking met kreatinine [13]. Tevens is de bepaling van cystatine C aanzienlijk duurder en de ervaring met kreatinine veel groter, waardoor cystatine C geen alternatief is voor kreatinine als marker voor GFR.

In de dagelijkse praktijk wordt de GFR geschat aan de hand van een formule gebaseerd op de kreatinine concentratie. De formule is opgebouwd uit de ratio lichaamslengte (LH; cm) van het kind en de plasma kreatinine concentratie (Pcr; µmol/L), vermenigvuldigd met een een constante (factor k):

$$GFR\ (mL/\mathrm{min}/1.73m^2) = \frac{k \times LH}{Pcr}$$

Voor k zijn in de loop van de tijd verschillende waarden gedefinieerd: k = 38 (Counahan et al.), k = 40 (Morris et al.) en k = 48.7 (Schwartz et al.) [14-16]. In de praktijk wordt vaak de waarde k = 40 gehanteerd. Hierbij dient te

worden opgemerkt dat het aan te raden is om lokaal de waarde voor k te bepalen, omdat de waarde samenhangt met de bepalingsmethode die als referentie wordt gebruikt voor het nauwkeurig volgen van de nierfunctie en de patiëntenpopulatie [17]. Men dient zich te realiseren dat de GFR berekend aan de hand van bovenstaande fomule te onnauwkeurig is om de nierfunctie nauwkeurig te volgen, zoals gewenst is bij bijvoorbeeld kinderen met progressieve nierinsufficiëntie of kinderen die een niertransplantatie hebben ondergaan. Pierrat et al. rapporteerde een overschatting van 20-25% voor de GFR berekend aan de hand van de formule ten opzichte van de urine klaring van inuline (n=198, leeftijd: 3-19 jaar) [18].
In een eigen onderzoek werd in meer dan 40% van de patiënten een verschil groter dan 10 mL/min/1.73m² tussen de GFR geschat aan de hand van bovenstaande formule en de inuline plasma klaring, bepaald zoals beschreven in dit artikel, gevonden (n=48, leeftijd: 5-18 jaar) [17].
Indien een nauwkeurige meting van de GFR gewenst is, gaat de voorkeur uit naar het bepalen van de plasma klaring van inuline met de single injectie methode met een beperkt aantal afnametijdstippen. Deze methode is praktischer en patiëntvriendelijker dan de 'gouden standaard' methode waarbij urineverzameling noodzakelijk is. In dit onderzoek werd de inuline single injectie methode voor het bepalen van de GFR bij kinderen gevalideerd.
Voor patiënten met een GFR < 75 mL/min/1.73m² gaf de inuline single injectie methode met 4 afnametijdstippen (10, 30, 90 en 240 min) een inuline plasma klaring, die gemiddeld 0.6 mL/min/1.73m2 lager was dan de inuline plasma klaring gebaseerd op 11 afnametijdstippen. Het gevonden verschil in inuline klaring was niet statistisch significant. Bij patiënten met een GFR > 75 mL/min/1.73m² was sprake van een lichte bias, die niet klinisch relevant was. Aangezien het met name bij een lage GFR van belang is dat een kleine verandering in nierfunctie (circa 10 mL/min/1.73m²) kan worden waargenomen, kan op grond van de gevonden resultaten worden geconcludeerd dat de inuline single injection methode met 4 afnametijdstippen een geschikte methode is voor het bepalen van de GFR bij kinderen. Zo kan met deze methode de nierfunctie nauwkeurig worden gemeten bij kinderen, bij wie door bijvoorbeeld afwijkende lichaamsproporties de kreatinine plasma concentratie niet betrouwbaar is. Tevens is het een relatief eenvoudige methode om de nierfunctie te bepalen bij bijvoorbeeld kinderen met progressieve nierinsufficiëntie of bij kinderen die een niertransplantatie hebben ondergaan.

see pages 88 - 89 for references

LITERATUURLIJST

1. Barratt TM, Avner ED, Harmon WE (1999) Pediatric Nephrology, 4e druk. Lippincott Williams & Wilkins, Baltimore, pp 351-352

2. Swinkels DW, Hendriks JCM, Nauta J, de Jong MCJW (2000) GFR by single-injection inulin clearance: definition of a workable protocol for children. Ann Clin Biochem 37:60-66

3. Van Rossum LK, Mathot RAA, Cransberg K, Vulto AG (2003) Optimal sampling strategies to assess inulin clearance in children by the inulin single-injection method. Clin Chem 49:1170-1179

4. Summerfield AL, Hortin GL, Smith CH et al. (1993) Automated enzymatic analysis of inulin. Clin Chem 39:2333-2337

5. Wong EC, Wilhite TR, Miller R et al. (1994) Less expensive enzymatic analysis for inulin with a kinetic assay. Clin Chem 40:1788-1789

6. Peck CC, Beal SL, Sheiner LB, Nichols AI (1984) Extended least squares nonlinear regression: a possible solution to the "choice of weights" problem in analysis of individual pharmacokinetic data. J Pharmacokinet Biopharm 12:545-58

7. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307-310

8. Rahn KH, Heidenreich S, Bruckner D (1999) How to assess glomerular function and damage in humans. J Hypertens 17:309-17

9. Hellerstein S, Berenbom M, Alon U, Warady BA (1993) The urinary clearance and infusion clearance of inulin are similar, but not identical. Kidney Int 44:1058-1061

10. Van Rossum LK, Cransberg K, De Rijke YB, Zietse R, Lindemans J, Vulto AG (2005) Determination of the inulin clearance by single injection or infusion in children. Ped Nephrol 20:777-781

11. Grubb A (1992) Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 38:S20-27

12. Randers E, Erlandsen EJ (1999) Serum cystatin C as an endogenous marker of the renal function--a review. Clin Chem Lab Med 37:389-395

13. Laterza OF, Price CP, Scott MG (2002) Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem 48:699-707

14. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM (1976) Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child 51:875-878

see pages 88 - 89 for references

15. Morris MC, Allanby CW, Toseland P, Haycock GB, Chantler C (1982) Evaluation of a height / plasma creatinine formula in the measurement of glomerular filtration rate. Arch Dis Child 57:611-615

16. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259-263

17. Van Rossum LK, Mathot RAA, Cransberg K, Zietse R, Vulto AG (2005) Estimation of the GFR in children: which algorithm should be used? Ped Nephrol 20:1769-1775

18. Pierrat A, Gravier E, Saunders C, Caira MV, Ait-Djafer Z, Legras B, Mallie JP (2003) Predicting GFR in children and adults: a comparison of the Cockcroft-Gault, Schwartz, and modification of diet in renal disease formulae. Kidney Int 64:1425-36

CHAPTER 5

ESTIMATION OF THE GLOMERULAR FILTRATION RATE IN CHILDREN: WHICH ALGORITHM SHOULD BE USED?

L.K. van Rossum[1], R.A.A. Mathot[1], K. Cransberg[2], R. Zietse[3], A.G. Vulto[1]

[1] Department of Hospital Pharmacy,
[2] Department of Pediatric Nephrology,
[3] Department of Internal Medicine,
Erasmus MC, Rotterdam, The Netherlands

Pediatric Nephrology, 2005;20:1769-1775

see pages 106 - 107 for references

ABSTRACT

Glomerular filtration rate (GFR) in children can be estimated by GFR = k x BH / Pcr (BH: body height (cm); Pcr: plasma creatinine concentration (μmol/L)). For k, several values have been reported: k = 38 (Counahan), 40 (Morris) and 48.7 (Schwartz). In this study the predictive performance of these formulae was compared with that of newly developed formulae.

GFR measurements based on inulin concentration time curves were divided into an index (n=58) and a validation data set (n=48). In the index data set a value for k was derived by application of nonlinear mixed-effect modeling. This approach was also used to develop a formula that better explained the relationship between patient factors and GFR. Bias and precision of all formulae were calculated for the validation data set.

In the index data set a value of 41.2 was found for k, which was close to the value k = 40 (Morris). Both formulae estimated GFR well (bias< 5%; precision: 25%). Further modeling of the relationship between patient factors and GFR did not improve the predictive performance.

In our hospital GFR was best estimated by the formula with k = 40 and 41.2. It is recommended to assess the optimal value for k locally.

INTRODUCTION

In clinical practice the glomerular filtration rate (GFR) is determined to evaluate renal function in suspected renal diseases and to monitor renal function during treatment of renal diseases. The urinary clearance of inulin with continuous intravenous infusion of inulin is regarded as the gold standard to determine GFR in children [1]. However, this method requires an intravenous infusion of inulin and timed urine collections over a period of several hours, which is cumbersome. In smaller children it also requires bladder catheterization. For daily practice formulae using the creatinine plasma concentration are widely employed to estimate GFR in children [2]. Estimates for GFR can be obtained by multiplication of the ratio of the child's body height (BH; cm) and the plasma creatinine concentration (Pcr; µmol/L) by a constant (k) [3-5]:

$$GFR = \frac{k \times BH}{Pcr} \quad \textit{(formula A)}$$

Various values of k have been reported: k = 38 (Counahan et al.), k = 40 (Morris et al.) and k = 48.7 (Schwartz et al.). Schwartz et al. differentiated various values of k for infants and children of different ages, since the relationship between muscle mass and body height changes with age [6-8].
Most formulae for estimation of GFR have been created by using linear regression (plotting the reciprocal of creatinine concentration against the measured GFR). Interestingly, Léger et al. have recently used nonlinear mixed-effect modeling (NONMEM) to assess the relationship between patient factors and the plasma clearance of ^{51}Cr-EDTA in 64 children [9]. In NONMEM both fixed effects (i.e. relationships between pharmacokinetic parameters and patient factors) and random effects (i.e. inter- and intra-patient variability) are evaluated.
In the current study an algorithm was developed based on NONMEM analysis of inulin plasma concentration in pediatric patients. Using this population approach a k-value was derived for the formula as mentioned above (formula A). Also, it was investigated whether estimation of GFR could be improved by including more patient factors in the algorithm. The predictive performance (i.e. bias and precision) of the newly developed algorithms was compared with the formulae reported earlier by Counahan et al., Morris et al. and Schwartz et al.

see pages 106 - 107 for references

METHODS

Patients

All patients were seen at the outpatient clinic of the Erasmus MC - Sophia Children's Hospital of Rotterdam for routine determination of the renal function in the period April 2000 and August 2004.
One group of pediatric patients (index data set; n=42; 58 studies) was used to determine the optimal value for k and to develop a formula that better explained the relationship between patient factors and GFR. The second group of pediatric patients (validation data set; n=35; 48 studies) was applied to compare the predictive performance of the newly developed algorithms with the earlier reported formulae.

Study design

In all patients the single injection method was applied to determine the plasma clearance of inulin [10, 11]. This method is used as standard method for routine determination of the renal function in the Sophia Children's Hospital. Inulin (polyfructosan, Inutest®, Fresenius Pharma, Graz, Austria) was infused in a dose of 5000 mg/1.73 m² body surface area (maximum dose 5000 mg), at a constant rate over 30 seconds. After administration of inulin at least 4 blood samples were taken during a time window of 240 minutes after injection (10 / 30 / 90 / 240 minutes after injection). Inulin plasma clearance values were obtained by fitting of the individual curves to a two-compartment model using extended least squares estimation in NONMEM [12]. GFR was corrected for body surface area (mL/min/1.73m²).

Biochemical assay

The inulin plasma concentration was measured enzymatically on the Hitachi 912 (Roche Diagnostics) [13, 14]. The coefficient of variation for this method was 6.9% at 136 mg/L and 1.9% at 725 mg/L. The assay was linear up to 2100 mg/L. The creatinine plasma concentration was measured enzymatically on the Hitachi 912 (Roche Diagnostics).

Index data set: development of an algorithm to estimate GFR

Algorithms for GFR were derived by performing a population pharmacokinetic analysis in the index data set using the nonlinear mixed-effect modeling program NONMEM (double precision; version V, level 1.1). The pharmacokinetics of inulin was described on the basis of a 2-compartment model. Inulin pharmacokinetic parameters of the model were estimated in terms of clearance (CL), central volume of distribution (V_1), peripheral volume of distribution (V_2) and intercompartmental clearance (Q). A proportional error model was used to describe the inter-individual variability. The residual intra-individual variability was modeled with a combined additive-proportional error model. An estimate for k in formula A was obtained by modeling the inulin plasma concentration time profiles of the index data set. In a more

extensive analysis the relationship between CL (i.e. GFR) and the following factors was tested: Pcr (µmol/L), age (AGE, years), body surface area (BSA, m^2), body weight (WT, kg), height (HT, cm), and sex (male / female). In the basic NONMEM model all factors were introduced simultaneously using:

$$CL=\theta 1 \times \left(\frac{Pcr}{medianPcr}\right)^{\theta 2} \times \left(\frac{AGE}{medianAGE}\right)^{\theta 3} \times \left(\frac{BSA}{medianBSA}\right)^{\theta 4} \times \left(\frac{WT}{medianWT}\right)^{\theta 5} \times \left(\frac{HT}{medianHT}\right)^{\theta 6} \times \theta 7^{sex}$$

(**formula B**), in which θ1 is the clearance of a female patient with median Pcr, AGE, BSA, WT and HT, and θ2, θ3, θ4, θ5 and θ6 are exponentials. θ7 is the fractional increase of clearance in males (i.e. SEX=1) when compared to females (i.e. SEX=0). The patient factors were separately eliminated from the basic model. A factor was retained when exclusion significantly ($p<0.01$) worsened the fit of the model. The main criterion of decision was the likelihood ratio test [15].

Validation data set: predictive performance of the formulae to estimate GFR

Agreement between the estimated GFR and the plasma clearance of inulin was evaluated by calculating the predictive performance and creating Bland-Altman plots for the validation data set. The predictive performance includes the mean relative prediction error (MPE%) and its 95% confidence interval as a measure of bias, and the root mean squared relative prediction error (RMSE%) and its 95% confidence interval as a measure of imprecision [16]. MPE%, RMSE% and the standard error for MPE% were defined as follows:

$$MPE\% = \frac{\sum_{i=1}^{n} pe_i}{n} \times 100\%$$

$$SE\% = \sqrt{\frac{\sum_{i=1}^{n} (pe_i - MPE)^2}{n \times (n-1)}} \times 100\%$$

$$RMSE\% = \sqrt{\frac{\sum_{i=1}^{n} (pe_i)^2}{n}} \times 100\%$$

in which n is the number of clearance pairs (i.e. reference and estimated values) and pe is the prediction error (pe = ln (GFRestimated) - ln (GFRinulin)). The log transformation was performed to avoid bias in favour of high values. Ninety five percent confidence interval of RMSE% was obtained by calculating 95% confidence interval of mean squared relative prediction error and extracting the root. Ideally MPE is 0 and RMSE is < 30%.
For the Bland-Altman plot the difference in estimated GFR and inulin plasma clearance was plotted against the mean GFR as described by Bland and Altman [17]. The statistical analysis was made by using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA).

RESULTS

Patient characteristics are summarized in Table I. In the index dataset 14 patients were included twice and in 1 patient the renal function was determined 3 times, while 13 patients were included twice in the validation data set. These patients were included as new patients since GFR varied and the time between the measurements was at least 1 year.
In the basic population model (index data set) no correlation between CL and patient factors was assumed and the following population estimates were obtained: CL = 37.4 mL/min, V_1= 6.81 L, V_2 = 4.35 L and Q= 119 mL/min. Unexplained inter-patient variability for CL was 118%. Adding body height and plasma creatinine concentration as determinants for the plasma clearance of inulin in the population model (formula A) significantly improved the fit. The unexplained inter-patient variability was reduced to 24% and k was 41.2 ± 5.6.
Unexplained inter-patient variability was further reduced to 17% when all patient factors were included in the population model (formula B). Removal of the patient factors age (AGE), weight (WT), body surface area (BSA) and gender (SEX) did not significantly worsen the population model (unexplained inter-patient variability in clearance: 18%). For a patient with a median body height of 146 cm and a median plasma creatinine concentration of 118 µmol/L the clearance was 51.2 ± 1.9 mL/min/1.73m². Exponents for body height and plasma creatinine concentration were 0.489 ± 0.160 and -0.736 ± 0.071, respectively. Table II presents the formulae to estimate GFR. The predictive performance of the tested formulae for the validation data set is given in Table III. Figure 1 shows the 95% confidence interval of the bias and precision for the tested formulae in the validation data set.
GFR estimated by the formula developed from the index data set by NONMEM (formula B) showed a bias of –4.3 % and a precision of less than 30%. Using k = 41.2 resulted in GFR estimates which were not statistically different from the reference values (i.e. inulin plasma clearances). The formula according to Counahan et al. and Morris et al. was not significantly biased and the precision was around 25%.

Table I. Patient characteristics

	Index data set	Validation data set
Number of studies	58	48
Patients	42	35
male	27	23
female	15	12
Age (years)		
median	15	13
range	4-20	5-18
Weight (kg)		
median	45	47
range	13-89	20-82
Height (cm)		
median	150	152
range	97-179	106-186
BSA (m^2)		
median	1.35	1.44
range	0.59-2.05	0.75-1.98
Creatinine concentration (µmol/L)		
median	87	89
range	19-660	35-434
Plasma clearance of inulin (mL/min/1.73m^2)		
median	65	71
range	6-172	7-158
Disorders		
kidney transplantation	35	28
other disorders	7	7

BSA: Body Surface Area

see pages 106 - 107 for references

Table II. Formulae to estimate GFR

Earlier reported formulae:

Counahan et al.[3]	$GFR(mL/min/1.73m^2) = \frac{38 \times BH}{Pcr}$
Morris et al.[4]	$GFR(mL/min/1.73m^2) = \frac{40 \times BH}{Pcr}$
Schwartz et al.[5]	$GFR(mL/min/1.73m^2) = \frac{48.7 \times BH}{Pcr}$
Léger et al.[9]	$GFR(mL/min) = \frac{56.7 \times BW + 0.142 \times BH^2}{Pcr}$

Formulae developed from index data set:

Formula A	$GFR(mL/min/1.73m^2) = \frac{41.2 \times BH}{Pcr}$
Formula B	$GFR(mL/min/1.73m^2) = \frac{51.2 \times \left(\frac{BH}{146}\right)^{0.489}}{\left(\frac{Pcr}{118}\right)^{0.736}}$

BH: body height (cm); Pcr: plasma creatinine concentration (μmol/L); BW: body weight (kg)

Table III. Predictive performance of the formulae to estimate GFR

	Validation data set (n=48)
Earlier reported formulae:	
Counahan et al. (k = 38)[3]	
Bias (%) [95% CI]	-5.9 [–13,1.5]
Imprecision (%) [95% CI]	26 [20,31]
Morris et al. (k = 40)[4]	
Bias (%) [95% CI]	-1.1 [-8.5,6.4]
Imprecision (%) [95% CI]	25 [19, 31]
Schwartz et al. (k = 48.7)[5]	
Bias (%) [95% CI]	19 [11, 26]
Imprecision (%) [95% CI]	31 [23, 38]
Léger et al.[9]	
Bias (%) [95% CI]	19 [11, 27]
Imprecision (%) [95% CI]	34 [25, 41]
Formulae developed from index data set:	
Formula A (k = 41.2)	
Bias (%) [95% CI]	1.9 [-5.5,9.3]
Imprecision (%) [95% CI]	25 [18,31]
Formula B (NONMEM)	
Bias (%) [95% CI]	-4.3 [-12,3.9]
Imprecision (%) [95% CI]	28 [19,36]

Bias: mean relative prediction error (MPE)

Imprecision: root mean squared relative prediction error (RMSE)

see pages 106 - 107 for references

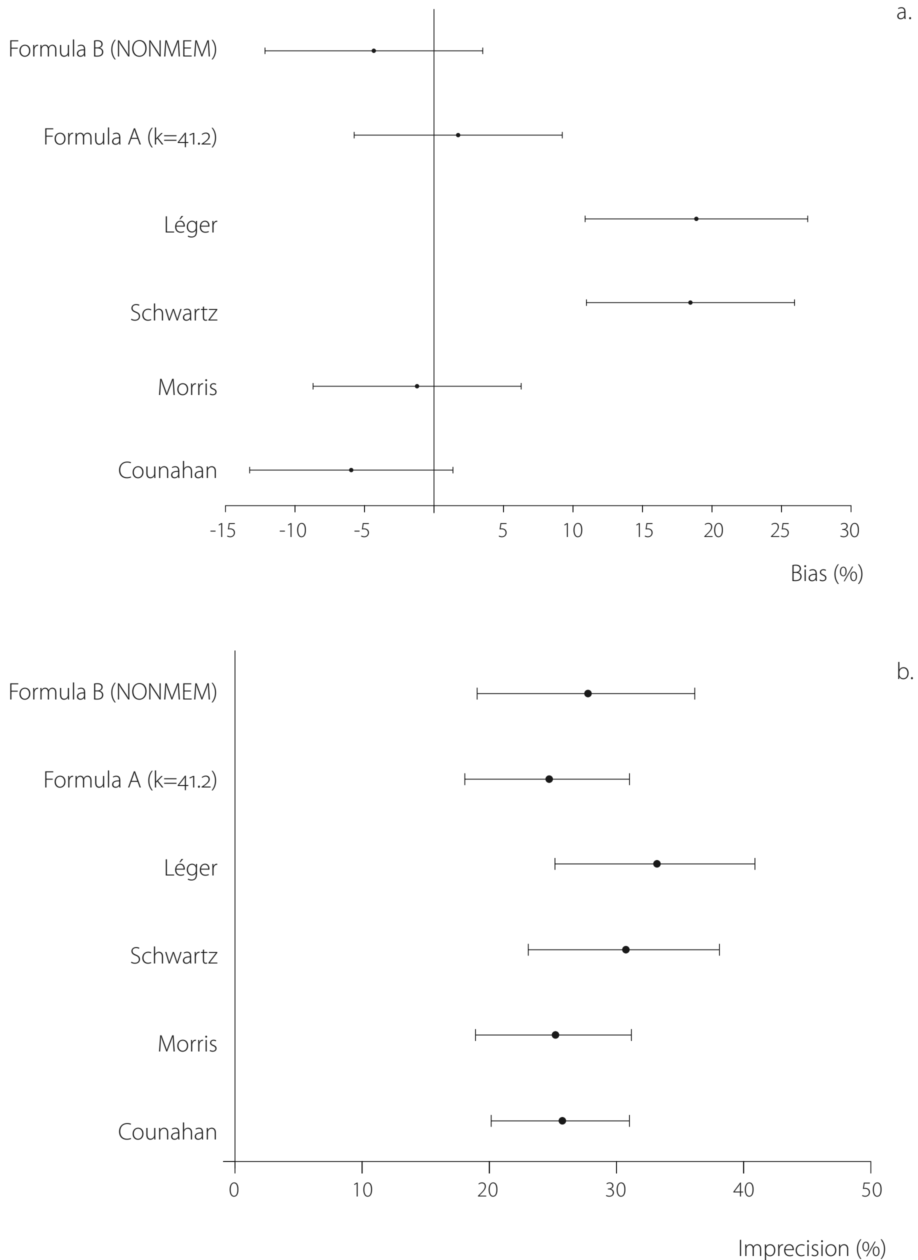


Figure 1

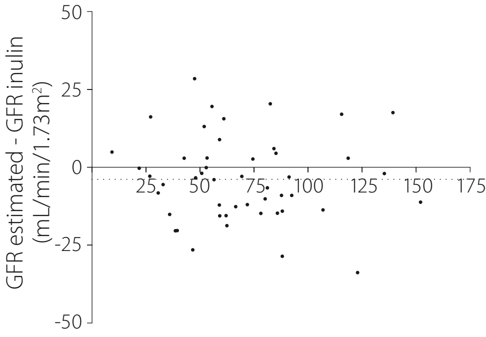
95% Confidence interval of the bias (a) and imprecision (b) for the tested formulae in the validation data set (n=48).

In contrast, the formula of Schwartz et al. showed a significant bias (bias: 19%; 95% CI: 11-26). Also, a significant overestimation was observed for the formula according to Léger et al. (bias: 19%; 95% CI: 11-27).
Figure 2 shows the Bland-Altman plots for the estimated GFR and the inulin plasma clearance. The mean difference between estimated and reference GFR varied from −0.97 for the formula of Morris et al. to 15 mL/min/1.73m² for the formula according to Léger et al. For all the formulae it was remarkable that the difference between estimated GFR and the reference inulin plasma clearance was larger than 10 mL/min/1.73m² in more than 20 patients (range: 44-63%, Table IV).

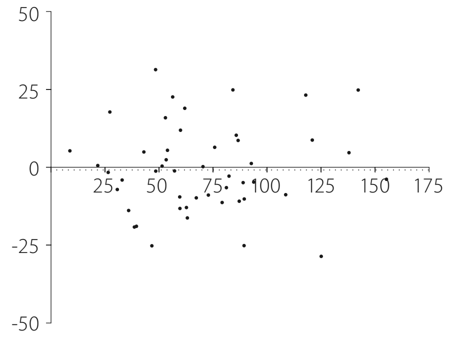
Table IV. Number of patients in the validation data set (n=48) with a difference between the estimated GFR and reference GFR (i.e. inulin plasma clearance) > 10 mL/min/1.73m²

	Number of patients (%)
Earlier reported formulae:	
Counahan et al.[3]	26 (54)
Morris et al.[4]	22 (46)
Schwartz et al.[5]	30 (63)
Léger et al.[9]	28 (58)
Formulae developed from index data set:	
Formula A (k = 41.2)	21 (44)
Formula B (NONMEM)	29 (60)

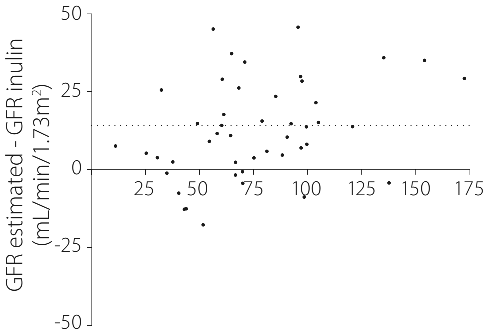
see pages 106 - 107 for references



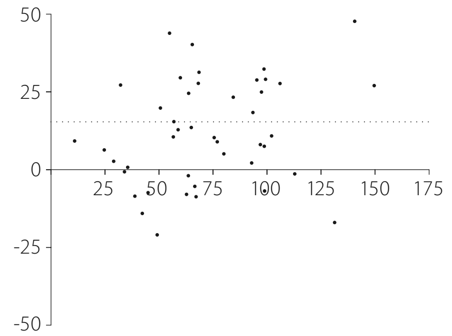
Formula according to Counahan et al. (k = 38)



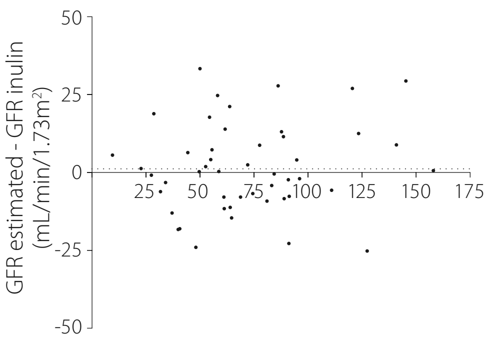
Formula according to Morris et al. (k = 40)



Formula according to Schwartz et al. (k = 48.7)

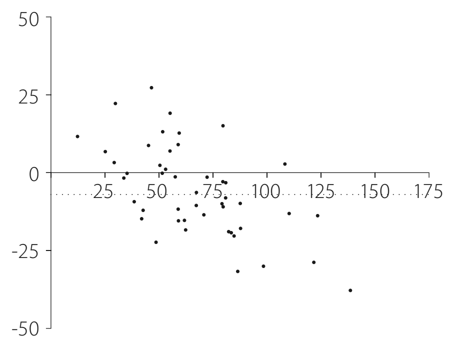


Formula according to Léger et al.



Formula A (k = 41.2)

Mean GFR (mL/min/1.73m²)



Formula B (NONMEM)

Mean GFR (mL/min/1.73m²)

Figure 2

Bland-Altman plots of the estimated GFR (GFR estimated) and the reference GFR (inulin plasma clearance, GFR inulin) (··· mean difference).

DISCUSSION

In daily clinical practice formulae based on the creatinine plasma concentration are widely employed to estimate GFR in children. Estimates for GFR can be obtained by multiplication of the ratio of the child's body height and the plasma creatinine concentration by a constant k. In this study an algorithm was developed based on NONMEM analysis of inulin plasma concentration in pediatric patients.
The predictive performance of the newly developed algorithms was compared with the earlier reported formulae.
The formula with k = 41.2 and the formula of Morris et al. (k = 40) resulted in the best predictive performance with a bias less than 5% and a precision of around 25%. The formula developed from the index data set by more extensive evaluation of patient factors did not produce a better predictive performance. Application of the formulae of Schwartz et al. (k = 48.7) and Léger et al. yielded a significant overestimation of GFR.
The formula of Counahan et al. and Morris et al. resulted in a better predictive performance in comparison with the formula according to Schwartz et al. The former formulae are based on the plasma clearance of ^{51}Cr-EDTA, while the formula according to Schwartz et al. was derived from the urinary clearance of creatinine.
To calculate the predictive performance of the formulae in the validation data set, the inulin plasma clearance was used as reference clearance. In general, the plasma clearance of ^{51}Cr-EDTA with single injection agreed well with the plasma clearance of inulin [18], while the urinary clearance of creatinine overestimated the inulin clearance especially at low levels of GFR due to tubular secretion [19, 20]. So it was expected that the formula of Morris et al. and Counahan et al. produced a better estimate of GFR than the formula according to Schwartz et al. The formula of Morris et al. predicted GFR better than the formula according to Counahan et al. (bias −1.1 vs −5.9%). Differences in patients' characteristics or variation in the method for analysis, can possibly account for this difference.
The formula developed from the index data set by more extensive evaluation of patient factors (NONMEM-analysis) produced a minor predictive performance compared with the formula based on body height, plasma creatinine concentration and the constant k = 40 or 41.2. This finding is not in agreement with our expectation, since it was suggested that including more patient factors results in a higher predictive performance. However, for daily practice it is very advantageous that a less complicated formula can be used to estimate GFR.
The formula according to Léger et al. overestimated the inulin plasma clearance.

see pages 106 - 107 for references

This can be attributed to the use of different methods to measure GFR. In that study the ^{51}Cr-EDTA plasma clearance was used as reference method, while we applied the inulin plasma clearance. However, good agreement between the inulin and the ^{51}Cr-EDTA plasma clearance was reported, but only if a two-compartment model was applied to construct the plasma concentration-time decay curve of ^{51}Cr-EDTA [18]. In the study of Léger et al. a one-compartment model was used. Aperia et al. demonstrated that a one-compartment analysis of ^{51}Cr-EDTA plasma clearance resulted in a significantly higher GFR. Theoretically it is possible that if the formula according to Léger et al. was based on a two-compartment analysis the predictive performance of that formula would have been better.

Since many years the impact of creatinine assays on the difference between estimated GFR and reference GFR has been discussed in numerous articles. Calibration bias in measuring serum creatinine concentration as well as pseudo-creatinine contribution of proteins (protein error) has been described [21, 22]. In our study the creatinine concentration was measured enzymatically, while the creatinine concentration in the studies of Morris et al, Counahan et al., Schwartz et al. and Léger et al. was measured non-enzymatically. As a consequence we could not exclude calibration bias. The protein error was eliminated by using an enzymatic method for creatinine measuring.

Several studies have been published describing formulae to estimate GFR in different patient populations. Paap et al. compared 10 formulae to estimate GFR with the 24-h urinary clearance of creatinine in children with varying degree of renal dysfunction (n=22, 7-16 years of age) [23]. Formulae based on the ratio of the child's body height and the plasma creatinine concentration, multiplied by a constant k, were tested, as well formulae which are applied in adults (for example formula of Cockroft and Gault). The formula with k = 46 had the best predictive performance, although the predictive performance was worse in patients with a lower GFR (GFR <30 mL/min/1.73m²). Paap et al. found a higher value for k since the urinary clearance of creatinine was used as reference method. As mentioned above the urinary clearance of creatinine overestimated the inulin clearance. As a consequence a higher value for k is required. Recently, Hellerstein et al. compared the formula of Léger et al. with the formula based on the ratio of the child's body height and the plasma creatinine concentration. The optimal value for k was determined locally (k = 44 for girls and boys younger than 13 years and k = 52 for boys older than 13 years; n=216, 4.8-21 years of age) [24]. The formula of Léger et al. was not superior to the GFR calculated with k = 44 or 52. However, in the study of Hellerstein et al. the values for k were derived from and tested in the same data set, which is statistically incorrect. An independent data set has to be used for validation, since using one data set for developing and testing always results in a good performance. We applied an

index data set to develop the algorithms and tested the algorithms by calculating the predictive performance in the validation data set.

The present study was performed in patients older than 4 years and most of them had a kidney transplant. It is not clear what the effect of this is on the optimal value of k. Theoretically it is possible that for patients without a kidney transplant the optimal k value differs. It would be interesting to evaluate the formula with k = 40 and 41.2 in children without a kidney transplant.

We only tested the constant k = 48.7 in the formula according to Schwartz et al. and did not apply a higher value of k with increasing age, since for daily practice it is preferred to use one value of k for all ages. A second reason why only k = 48.7 was tested, was that applying various values for k also resulted in an overestimation of GFR (n=198, 3-19 years of age, mean difference in GFR: 20 mL/min/1.73m²) [25]. Furthermore, GFR estimated by the formula with k = 48.7 showed already an overestimation, this overestimation would only be larger if for boys older than 13 years of age a higher value of k (k = 61.9) was applied.

One has to realize that the formulae to estimate GFR can only be applied for clinical daily practice. The formulae are too imprecise for an accurate determination of GFR as desired in a research setting. In trials designed to improve graft function it is important to be able to detect small improvements in GFR (for example 10 mL/min/1.73m²). Pierrat et al. reported an overestimation of 20-25% for the formula according to Schwartz et al. [25]. We found that the difference between estimated GFR and inulin plasma clearance for all formulae was larger than 10 mL/min/1.73m² in more than 40% of the patients in the validation data set. Therefore the formulae cannot replace the classical method for determination of GFR.

In conclusion, in this study GFR was best estimated by the formula with a k value of 40 and 41.2. Introduction of more patient factors resulted in a formula, which was not better than the formula with a k value of 40 or 41.2. However, it is important to realize that these results are closely linked with the method, which was used for an accurate determination of GFR (reference method), and the population of patients. As a consequence it is recommended to assess the optimal value for k locally.

see pages 106 - 107 for references

REFERENCES

1. Barratt TM, Avner ED, Harmon WE (1999) Pediatric Nephrology, 4th edn. Lippincott Williams & Wilkins, Baltimore, pp 351-352

2. Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ et al. (2003) National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. Pediatrics 111:1416-21

3. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM (1976) Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child 51:875-878

4. Morris MC, Allanby CW, Toseland P, Haycock GB, Chantler C (1982) Evaluation of a height / plasma creatinine formula in the measurement of glomerular filtration rate. Arch Dis Child 57:611-615

5. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259-263

6. Schwartz GJ, Feld LG, Langford DJ (1984) A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr 104:849-854

7. Schwartz GJ, Brion LP, Spitzer A (1987) The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am 34:571-590

8. Schwartz GJ (1992) Does kL/PCr estimate GFR, or does GFR determine k? Pediatr Nephrol 6:512-515

9. Leger F, Bouissou F, Coulais Y, Tafani M, Chatelut E (2002) Estimation of glomerular filtration rate in children. Pediatr Nephrol 17:903-907

10. Van Rossum LK, Mathot RAA, Cransberg K, Vulto AG (2003) Optimal sampling strategies to assess inulin clearance in children using the inulin single injection method. Clin Chem 49:1170-1179

11. Swinkels DW, Hendriks JC, Nauta J, de Jong MC (2000) Glomerular filtration rate by single-injection inulin clearance: definition of a workable protocol for children. Ann Clin Biochem 37:60-66

12. Peck CC, Beal SL, Sheiner LB, Nichols AI (1984) Extended least squares nonlinear regression: a possible solution to the "choice of weights" problem in analysis of individual pharmacokinetic data. J Pharmacokinet Biopharm 12:545-58

see pages 106 - 107 for references

13. Summerfield AL, Hortin GL, Smith CH, Wilhite TR, Landt M (1993) Automated enzymatic analysis of inulin. Clin Chem 39:2333-2337

14. Wong EC, Wilhite TR, Miller R, Smith CH, Landt M (1994) Less expensive enzymatic analysis for inulin with a kinetic assay. Clin Chem 40:1788-1789

15. White DB, Walawander CA, Liu DY, Grasela TH (1992) Evaluation of hypothesis testing for comparing two populations using NONMEM analysis. J Pharmacokinet Biopharm 20:295-313

16. Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokin Biopharm 9:503-51

17. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307-310

18. Aperia A, Freyschuss U (1984) Comparison of plasma clearances of polyfructosan and 51Cr-EDTA in children. Acta Paediatr Scand 73:379-382

19. Arant BS Jr, Edelmann CM Jr, Spitzer A (1972) The congruence of creatinine and inulin clearances in children: use of the Technicon AutoAnalyzer. J Pediatr 81:559-561

20. Hellerstein S, Alon U, Warady BA (1992) Creatinine for estimation of glomerular filtration rate. Pediatr Nephrol 6:507-511

21. Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, Levey AS (2002) Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. Am J Kidney Dis 39:920-9

22. Wuyts B, Bernard D, Van den Noortgate N, Van de Walle J, Van Vlem B, De Smet R, De Geeter F, Vanholder R, Delanghe JR (2003) Reevaluation of formulas for predicting creatinine clearance in adults and children, using compensated creatinine methods. Clin Chem 49:1011-4

23. Paap CM, Nahata MC (1995) Prospective evaluation of ten methods for estimating creatinine clearance in children with varying degrees of renal dysfunction. J Clin Pharm Ther 20:67-73

24. Hellerstein S, Berenbom M, DiMaggio S, Erwin P, Simon SD, Wilson N (2004) Comparison of two formulae for estimation of glomerular filtration rate in children. Pediatr Nephrol 19:780-4

25. Pierrat A, Gravier E, Saunders C, Caira MV, Ait-Djafer Z, Legras B, Mallie JP (2003) Predicting GFR in children and adults: a comparison of the Cockcroft-Gault, Schwartz, and modification of diet in renal disease formulae. Kidney Int 64:1425-36

CHAPTER 6

RENAL EXTRACTION OF CYSTATIN C VERSUS ^{125}I-IOTHALAMATE IN HYPERTENSIVE PATIENTS

L.K. van Rossum[1], R. Zietse[2], A.G. Vulto[1], Y.B. de Rijke[3]

[1] Department of Hospital Pharmacy,
[2] Department of Internal Medicine,
[3] Department of Clinical Chemistry,
Erasmus MC, Rotterdam, The Netherlands

Nephrology Dialysis Transplantation: submitted

see pages 116 - 117 for references

ABSTRACT

Several markers are available to estimate the glomerular filtration rate (GFR) in patients. Cystatin C is a relatively new marker and has been suggested as an alternative for creatinine. Numerous studies have been performed to evaluate the usefulness of cystatin C to estimate GFR. The aim of this study was to compare the renal extraction of cystatin C with that of ^{125}I-iothalamate in hypertensive patients.

Forty hypertensive patients with unilateral renal artery stenosis, and who used at least two antihypertensive agents, were studied. For the determination of the renal extraction ratio, blood samples were drawn simultaneously from the renal vein and the abdominal aorta. The renal extraction ratio was calculated as ([A]-[V]) / [A], in which A is the plasma concentration of the compound from the abdominal aorta, and V is the plasma concentration of the compound from the renal vein.

The mean difference between the renal extraction ratio of cystatin C and that of ^{125}I-iothalamate was 0.002. The 95% confidence interval for the mean difference was -0.036 to 0.032, which was not statistically significant. However, the limits of agreement were large (–0.271 and 0.267).

The mean renal extraction of cystatin C was in agreement with the mean renal extraction of ^{125}I-iothalamate in hypertensive patients, however a very large variation was found. Therefore, the use of cystatin C to estimate GFR is limited.

INTRODUCTION

The ability to measure renal function accurately is essential for evaluating patients with suspected renal diseases and for studying changes in renal function. Several markers are available to estimate the glomerular filtration rate (GFR). Creatinine is widely used to estimate GFR. It is a metabolic product of creatine and phosphocreatine in the muscle and its production is proportional to the total muscle mass. This leads to a variation in serum creatinine concentration across age, gender, race, nutritional status and body composition that is independent from changes in GFR. Moreover, creatinine is not only filtered by the glomerulus, but also secreted by the proximal tubule. As a consequence the GFR based on the plasma creatinine concentration is overestimated, particularly at lower GFR.
As a result, studies with alternative markers for the estimation of GFR have been performed. Cystatin C is a relatively new marker and has been suggested as an alternative for creatinine [1, 2]. Cystatin C is a proteinase inhibitor with a molecular weight of 13.3 kDa and is produced by all nucleated cells. It is freely filtered by the glomerular membrane, reabsorbed and completely metabolized in the proximal tubule and does not return to the circulation [3]. Several studies have been performed to evaluate the usefulness of cystatin C to estimate GFR [3-7].
As cystatin C is metabolized in the tubule, it is not possible to determine the urinary clearance of cystatin C. Therefore, we applied the renal extraction ratio of cystatin C to evaluate whether cystatin C is a useful to estimate GFR. The renal extraction ratio is a parameter for renal function and expresses the glomerular filtration and tubular handling (tubular secretion and / or reabsorption) of a substance.
The aim of this study was to compare the renal extraction of cystatin C with that of ^{125}I-iothalamate in hypertensive adults.

METHODS

Patients

We studied 40 consecutive patients with suspected unilateral renal artery stenosis. All patients had a diastolic blood pressure higher than 95 mmHg and used at least two antihypertensives. The study was approved by the hospital review board and done during the diagnostic work-up for renal artery stenosis. Renal function was measured using the constant infusion clearance technique of ^{125}I-iothalamate without urine collections [8]. For the determination of the renal extraction ratio

see pages 116 - 117 for references

blood samples were drawn simultaneously from the renal vein and the abdominal aorta in sodium citrate tubes; first at one side and immediately thereafter on the other. Before each blood sampling the correct positioning of the catheter in the renal vein was confirmed by X-ray control and oxygen saturation measurement.
The renal extraction ratio of cystatin C (Ecyst C) and ^{125}I-iothalamate (Ethal) were calculated as ([A]-[V]) / [A], in which A is the plasma concentration of the compound from the abdominal aorta, and V is the plasma concentration of the compound from the renal vein.

Biochemical assay

Plasma samples were stored at -20°C until analysis. Cystatin C was measured in triplicate using a fully automated particle enhanced immuno turbidimetric method (DAKO Cystatin C PET kit, Copenhagen, Danmark). The assay was performed on a Hitachi 912 auto-analyzer (Roche Diagnostics, Basel, Switzerland). Inter- and intra-assay variation, calculated from the control samples with assigned values of 1.4 and 2.8 mg/L, was 11.3 and 5.6%, respectively. A concentration of triglycerides > 4 mmol/L interferes with the assay for cystatin C, therefore the triglycerides concentration was measured in all samples.

Statistical analysis

Data are presented as mean ± SD. Agreement between Ecyst C and Ethal was evaluated as described by Bland and Altman [9]. The limits of agreement represent the range around the mean difference between both methods in which 95% of the values will be found (mean difference ± 1.96xSD). The 95% confidence intervals of the mean difference were calculated by the mean difference ± *t x* standard error of the mean difference, in which *t* is the appropriate point of the *t* distribution with n-1 degrees of freedom.
The statistical analysis was made by using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA).

RESULTS

Forty hypertensive patients with unilateral renal artery stenosis were studied (male / female: 21 / 19; mean age: 55 ± 13 years).
All plasma samples had a triglycerides concentration lower than 4 mmol/L.
The mean plasma clearance of ^{125}I-iothalamate was 61 ± 21 mL/min/1.73m² and ranged from 27 to 108 mL/min/1.73m². For 1 patient the plasma clearance of ^{125}I-iothalamate was not available. In Figure 1 the relationship between the reciprocal cystatin C concentration and the plasma clearance of ^{125}I-iothalamate is shown. The squared correlation coefficient was 0.538.
In total 80 samples of the left and right kidney from 40 patients were available to determine the renal extraction ratio of cystatin C.
In 16 cases the sample volume was insufficient for analysis in triplicate. The range of renal extraction ratios varied from 0.01 to 0.54 for cystatin C and 0.01 to 0.33 for ^{125}I-iothalamate.

Figure 2 shows the Bland-Altman plot for Ecyst C and Ethal. Although the mean difference between Ecyst C and Ethal was small (0.002), the limits of agreement for the mean difference were large (–0.271 and 0.267). The 95% confidence interval for the mean difference was -0.036 to 0.032, which shows that the mean difference was not statistically significant.
From the first 20 patients the extraction ratio of creatinine was also available.
Figure 3 represents the Bland-Altman plot for the renal extraction ratio of creatinine (Ecreat) and ^{125}I-iothalamate (36 samples; mean difference: 0.014; 95% confidence interval of the mean difference: -0.011 to 0.039; limits of agreement: -0.131 and 0.159).

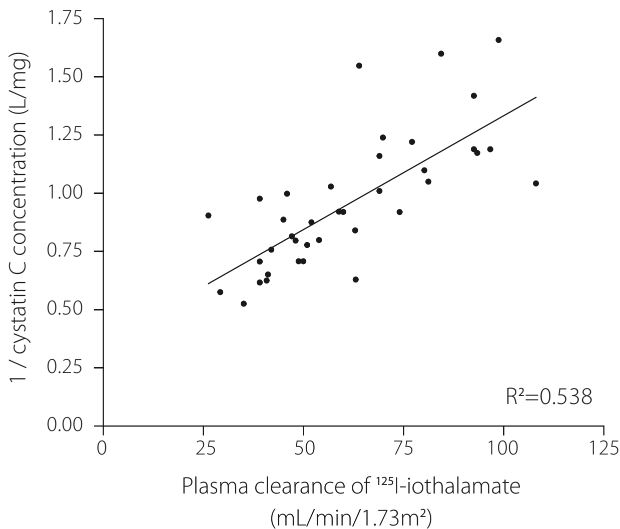


Figure 1

Correlation between the reciprocal cystatin C concentration and the plasma clearance of ^{125}I-iothalamate (39 patients).

see pages 116 - 117 for references

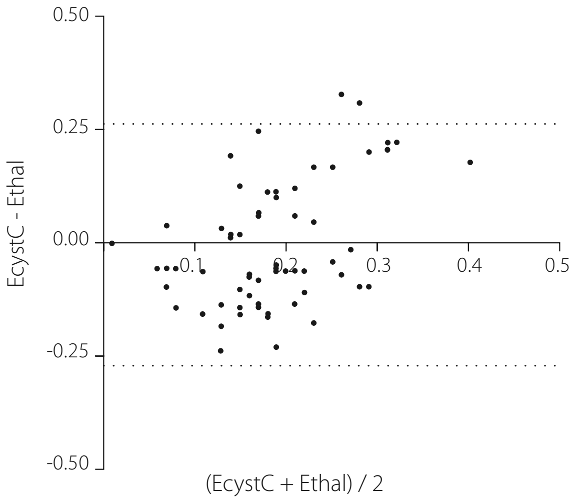


Figure 2

Bland-Altman plot of the renal extraction ratio of cystatin C and that of ^{125}I-iothalamate (··· limits of agreement) (64 samples from 40 patients).

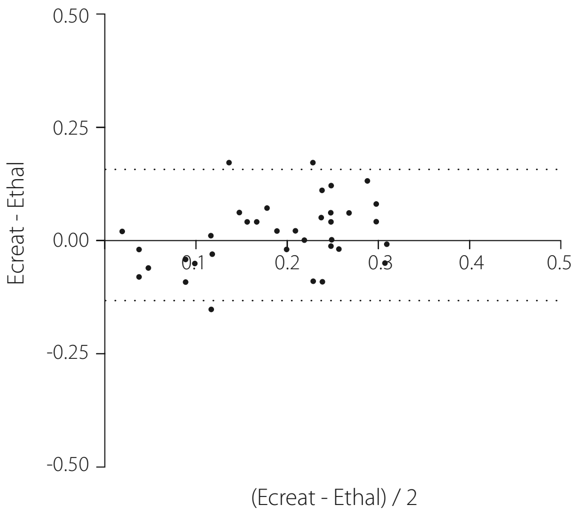


Figure 3

Bland-Altman plot of the renal extraction ratio of creatinine and that of ^{125}I-iothalamate (··· limits of agreement) (36 samples from 20 patients).

DISCUSSION

This study represented the first direct measurement of renal extraction of cystatin C. We observed that the mean difference between the extraction ratios of cystatin C and ^{125}I-iothalamate was not statistically significant. However, the limits of agreement showed a large range. In general, the renal extraction ratio shows a larger variation than the GFR, since a small variation in concentration has a large impact on the value of the extraction ratio. As the cystatin C concentration was measured at least in triplicate, it seems that this argument could not fully explain the observed variation.

The large limits of agreement (i.e. variation in the renal extraction of cystatin C) suggest that the theory that cystatin C is readily filtered in the glomerulus (sieving coefficient of 1) and subsequently metabolized in the tubule is untrue. Given its molecular weight of 13.3 kD, which is greater than that of either creatinine (0,113 kD) or inulin (5.2 kD), the sieving coefficient of cystatin C may not equal 1. The observed relationship with the plasma clearance of ^{125}I-iothalamate would than depend on, varying, tubular secretion.

Following the introduction of cystatin C many positive results were reported. In contrast to creatinine, cystatin C showed a constant production rate. However, more recently it appears that the use of cystatin C to estimate GFR has also limitations [3]. We demonstrated that the renal extraction of cystatin C has a large variation. Several other investigators reported that the plasma concentration of cystatin C is not only altered by renal function but also by nonrenal factors. Changes in cystatin C concentration do not reflect changes in GFR in children with a renal transplant, which has been attributed to the use of (a large dose of) glucocorticoids [3, 10-13]. It has also been reported that the concentration of cystatin C in patients with various types of cancer was increased irrespective of renal function [14]. However, it is not clear if this increase in cystatin C concentration was attributable to increased production rate or to decreased elimination [14, 15]. Knight et al. performed a multivariate analysis to identify factors influencing the serum cystatin C concentration. Older age, male gender, greater weight, greater height, current cigarette smoking, and higher serum C-reactive protein levels were all independently associated with increased serum cystatin C concentrations [16]. Thyroid dysfunction also has an impact on plasma cystatin C concentration [17, 18].

see pages 116 - 117 for references

The present study was performed in hypertensive patients who used two or more antihypertensive agents. It is not clear whether similar results will be found in non-hypertensive patients, since its theoretically possible that antihypertensive agents (such as angiotensin converting enzyme inhibitors or angiotensin II antagonists) change the renal extraction of cystatin C. However, we compared the renal extraction ratio of cystatin C with that of ^{125}I-iothalamate and the effect of the antihypertensive agents on glomerular filtration would equally affect both markers. Moreover, Knight et al. studied the effect of several factors on the serum cystatin C concentration and found that the presence of hypertension was not significantly associated with an increase in cystatin C concentration [16]. Therefore, the results can be extrapolated to other patient populations.

We conclude that the mean renal extraction of cystatin C was in agreement with the mean renal extraction of ^{125}I-iothalamate in hypertensive patients, however a very large variation was found. Therefore, the use of cystatin C to estimate GFR is limited.

REFERENCES

1. Grubb A, Simonsen O, Sturfelt G, Truedsson L, Thysell H (1985) Serum concentration of cystatin C, factor D and beta 2-microglobulin as a measure of glomerular filtration rate. Acta Med Scand 218:499-503

2. Simonsen O, Grubb A, Thysell H (1985) The blood serum concentration of cystatin C (gamma-trace) as a measure of the glomerular filtration rate. Scand J Clin Lab Invest 45:97-101

3. Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A (2005) Cystatin C as a marker of GFR--history, indications, and future research. Clin Biochem 38:1-8

4. Dharnidharka VR, Kwon C, Stevens G (2002) Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 40:221-6

5. Laterza OF, Price CP, Scott MG (2002) Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem 48:699-707

6. Newman DJ (2003) Cystatin C: what more do we need to know? Nephron Clin Pract 93: 122-123

7. Randers E, Erlandsen E (1999) Serum cystatin C as an endogenous marker of the renal function - a review. Clin Chem Lab Med 37:389-395

8. Zietse R, Blankestijn P, Bos B et al. (1995) Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. Clin Nephrol 43:29-34

9. Bland M, Altman D (1986) Statistical methods for assessing agreement between two methods of clinical measurements. Lancet 1:307-310

10. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J (1999) Cystatin C serum concentrations underestimate glomerular filtration rate in renal transplant recipients. Clin Chem 45:1866-1868

11. Bokenkamp A, van Wijk JA, Lentze MJ, Stoffel-Wagner B (2002) Effect of corticosteroid therapy on serum cystatin C and beta2-microglobulin concentrations. Clin Chem 48:1123-1126

12. Risch L, Huber AR (2002) Glucocorticoids and increased serum cystatin C concentrations. Clin Chim Acta 320:133-134

13. Risch L, Herklotz R, Blumberg A, Huber AR (2001) Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. Clin Chem 47:2055-2059

see pages 116 - 117 for references

14. Kos J, Stabuc B, Cimerman N, Brunner N (1998) Serum cystatin C, a new marker of glomerular filtration rate, is increased during malignant progression. Clin Chem 44:2556-2557

15. Newman DJ (1999) More on cystatin C. Clin Chem 45:718-719

16. Knight EL, Verhave JC, Spiegelman D et al. (2004) Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int 65:1416-21

17. Fricker M, Wiesli P, Brandle M, Schwegler B, Schmid C (2003) Impact of thyroid dysfunction on serum cystatin C. Kidney Int 63:1944-1947

18. Jayagopal V, Keevil BG, Atkin SL, Jennings PE, Kilpatrick ES (2003) Paradoxical changes in cystatin C and serum creatinine in patients with hypo- and hyperthyroidism. Clin Chem 49:680-681

CHAPTER 7

DISCUSSION

see pages 126 - 127 for references

DETERMINATION OF THE GLOMERULAR FILTRATION RATE

Renal function tests are important to evaluate the progression of kidney disease and to monitor renal function during drug treatment. For the assessment of renal function the mechanisms responsible for the formation of urine, namely filtration, reabsorption and secretion, must be taken into consideration. The excretion of endogenous and exogenous substances is mainly regulated by glomerular filtration. Glomerular filtration rate (GFR) is considered the most important parameter to evaluate renal function and it is affected by most kidney diseases.

The ideal marker for the determination of GFR is physiologically inert, freely filtered in the glomerulus and neither secreted, reabsorbed, synthesized, nor metabolized by the kidney. Inulin, an exogenous polysaccharide, has all of the properties of an ideal marker and is considered the gold standard. Other substances to determine GFR are radio-isotopes (for example ^{125}I-iothalamate, ^{51}Cr-EDTA and ^{99m}Tc-DTPA) and iohexol, all exogenous markers, and the endogenous markers creatinine and cystatin C.

GFR can be determined by measuring the appearance of a marker in the urine (urinary clearance) or the disappearance of the marker from the blood (plasma clearance). The urinary clearance is defined as the volume of plasma from which the marker shoud be totally cleared to account for its excretion in the urine during a certain period of time. It always requires the measurement of the urinary excretion rate. The urinary clearance is calculated by dividing the urinary excretion rate of the marker by its plasma concentration. Both endogenous and exogenous markers can be used for the determination of the urinary clearance. Critical aspects of this method are complete emptying of the bladder, an accurate measurement of the urine volume for each clearance period and a precise recording of the duration of the urine collection period. The plasma clearance can only be performed with an exogenous marker. The plasma clearance of an exogenous marker can be determined by use of either a continuous intravenous infusion or a single bolus injection. The continuous infusion method is based on the concept that when the plasma concentration of the marker is constant (steady state concentration) and the volume of distribution is saturated with the marker (state of equilibration), the rate of excretion equals the rate of infusion. GFR can be calculated from the infusion rate, the concentration of the marker in the infusate and the plasma concentration of the marker.

With the single injection method, a bolus injection of the marker is administered and blood samples are collected to construct a plasma concentration-time-decay curve.

The plasma clearance of the marker is calculated from the dose and the Area Under the concentration-time Curve (AUC), using a classical pharmacokinetic approach.

Both the National Kidney Foundation of

the United States and the European Renal Association - European Dialysis and Transplant Association developed clinical guidelines with recommendations for the clinical assessment of kidney diseases in adults and children [1, 2]. In order to determine GFR, the European guidelines recommend to use only methods, that have been validated in patients with advanced renal failure. According to these guidelines, the preferred method to determine GFR in advanced renal failure is the mean of urea and creatinine clearance (with 24 hour urine collection). However, creatinine is not only filtered by the glomerulus but also secreted by the renal tubules, while the renal tubules absorb urea. The mean of urea and creatinine clearance is close to GFR. The U.S. National Kidney Foundation states that the most widely applied method to determine GFR in clinical practice is based on the 24-hour creatinine clearance or serum creatinine concentration. The accuracy of urine-based GFR determination depends on the patient's ability to collect the urine properly over a defined time. Failing to empty the bladder at the start of the collection, failing to collect all urine passed during the collection interval and errors in timing the interval are common sources of error. Adults can be instructed to minimize these errors ('every drop of the urine has to be collected'), but in children it is cumbersome to collect the urine samples correctly. Bladder catheterization and making an ultrasonograph to check whether the bladder is empty can help, but such interventions are inconvenient to children. As a consequence, determination of the urinary clearance of a marker (for example creatinine) in children is not preferred.

Determination of the plasma clearance of a marker is an alternative method to determine GFR without collection of urine. The plasma clearance of inulin, radiolabelled markers (^{125}I-iothalamate, ^{51}Cr-EDTA, ^{99m}Tc-DTPA), non-radiolabelled iothalamate and iohexol can be determined in adults. Although inulin is considered as gold standard marker, in adults radiolabelled markers are commonly used since their measurement is relatively simple and very accurate. Potential safety drawbacks related to radiation exposure have led to a wide discussion around to use of these markers in children. Supporters of radiolabelled markers indicate that the exposure to radiation is limited and is not more than that of a radiograph. Opponents argue that one has to be careful with the use of radiolabelled markers in children and that there are good or even better alternatives (i.e. inulin). In general, the use of radiolabelled markers in children is limited. Therefore, the preferred marker to determine the plasma clearance in children is inulin and much experience has been gained with it in children.

see pages 126 - 127 for references

The plasma clearance of inulin can be measured by use of either a continuous intravenous infusion or a single bolus injection. In the Sophia Children's Hospital, an academic children's hospital with 270 beds, the inulin plasma clearance with continuous infusion has been used as the standard method to determine GFR for many years. With this method, hospitalization is necessary for the duration of the inulin infusion and it requires three capillary blood samples. This makes the procedure complex, invasive and time-consuming. The single injection method can be performed in daycare, with only one venous puncture for the administration of inulin and for the collection of blood samples. An inulin bolus is administered intravenously and blood samples are collected up to 240 min after injection. The inulin concentrations measured are used to construct a plasma concentration-time-decay curve. Ten to twelve blood samples are usually required for an accurate description of the curve. It is stressful for children to draw a lot of blood samples and therefore limited and optimal sampling strategies were introduced [3-5].

The single injection method with blood samples taken at 10, 30, 90 and 240 minutes after administration and Bayesian analysis had an excellent accuracy and was on average 9.7 mL/min/1.73m² higher than the inulin plasma clearance determined with the continuous infusion method [6].

ESTIMATION OF THE GLOMERULAR FILTRATION RATE IN DAILY PRACTICE

For daily practice formulae including the creatinine plasma concentration and patient's characteristics are widely employed to estimate GFR. Variation in creatinine production due to age- and sex-related differences in muscle mass has been incorporated in these formulae. It can be stated that the use of formulae including the creatinine concentration give more valid estimates of GFR than the serum creatinine concentration alone. The most frequently used formula to estimate GFR in adults is that of Cockroft and Gault, which includes age, body weight, gender and serum creatinine concentration [7]. This formula was developed in 1976 and was derived from the urinary creatinine clearance of 249 patients (mainly males; age: 18-92 years; mean GFR: 37 - 115 mL/min depending on age). More recently the MDRD (Modification of Diet in Renal Disease) formula was developed for adults [8]. This formula was derived from 1070 patients and validated in 558 patients with the urinary clearance of ^{125}I-iothalamate as reference method (mean age: 50.6 ± 12.7 years, mean GFR: 39.8 ± 21.2 mL/min/1.73m²). The original MDRD formula requires the variables serum creatinine, age, serum urea nitrogen, serum albumin, gender and race. An abbreviated version of the MDRD formula with serum creatinine, age, gender and race as variables has also been introduced, since the contributions of blood urea nitrogen

and serum albumin concentrations to the original formula were small [9, 10]. The Cockroft-Gault formula and the MDRD formula have been evaluated in different patient populations in numerous publications [1]. In general it can be said that the appropriateness of the formulae depends on the selected patients, the reference method to determine GFR and the assay to measure the serum creatinine concentration.
For children, estimates of GFR can be obtained by multiplying the ratio of the child's body height (cm) and the plasma creatinine concentration (µmol/L) by a constant k. Various values of k have been reported: k = 38 (Counahan et al.), k = 40 (Morris et al.) and k = 48.7 (Schwartz et al.) [11-13]. Recently, algorithms to estimate GFR in children were developed based on NONMEM analysis [14, 15]. However, these algorithms with extensive evaluation of patient factors did not produce a better predictive performance compared with the formula based on body height, plasma creatinine concentration and the constant k = 40 [15]. There is debate over which value to use for k. It is recommended to assess the optimal value for k locally, since the value of k is closely linked with the method used for an accurate determination of GFR (reference method), and the population of patients.

It is still unknown until which age a formula specially developed for children should be applied. As mentioned before the most common formulae to estimate GFR in children are based on the ratio of the child's body height (cm) and the plasma creatinine concentration (µmol/L) multiplied by a constant k. These formulae were derived from children aged from 2 months - 14 years (Counahan), 2 - 14 years (Morris) and 6 months - 20 years (Schwartz). When the Cockroft-Gault formula was developed patients aged from 18 - 92 years were included, while for the MDRD formula patients aged from 18 - 70 years were recruited. These formulae for adults and children show a small overlap with respect to age. Pierrat et al. compared the Cockroft-Gault formula, the MDRD formula and the Schwartz formula with different values for k with the urinary clearance of inulin in 198 children [16].
The children were divided into three age groups: < 8 years, 8 to 12 years and > 12 years. In children over 12 years old (n=116) the Cockroft-Gault formula was close to the inulin clearance (mean difference of GFR circa 15 ml/min/1.73m²), but the difference was significant. The MDRD and the Schwartz formulae overestimated GFR. Nevertheless, the authors concluded that the Cockroft-Gault formula could be used for children over 12 years of age. Paap et al. compared 10 formulae to estimate GFR with the 24-h urinary clearance of creatinine in children with varying degrees

see pages 126 - 127 for references

of renal dysfunction (n=22, 7-16 years of age) [17]. Formulae based on the ratio of the child's body height and the plasma creatinine concentration, multiplied by a constant k as well as the Cockroft-Gault formula were tested. The Cockroft-Gault formula was less accurate than formulae based on the ratio of the child's body height and the plasma creatinine concentration. Unfortunately, no stratification by age was applied. Recently Filler et al. compared the Cockroft-Gault formula and the formula of Schwartz (k = 38 for children younger than 13 years and girls older than 13 years; k = 48 for boys older than 13 years) with the renal clearance of ^{99m}Tc-DTPA in 262 children (1.0 - 18.9 years of age) [18]. A bias of - 19.0 ± 36.4% was found for the Cockroft-Gault formula compared with a bias of -12.8 ± 24.2% for the Schwartz formula in all patients. For boys older than 13 years both formulae showed a smaller bias (bias of 5.0 ± 23.5% for the Cockroft-Gault formula; bias of -6.8 ± 24.0% for the Schwartz formula with k = 48). It was not clear whether the GFR estimate by the Cockroft-Gault formula was corrected for body surface area.
In our data set (n=48) 5 patients had an age of > 16 years (n=4 aged 17 years and n=1 aged 18 years). In all these patients the Cockroft-Gault formula overestimated the inulin plasma clearance (mean estimated GFR was 87 mL/min/1.73m² vs mean reference GFR of 52 mL/min/1.73m²) and the abbreviated MDRD formula also produced an overestimation (mean estimated GFR: 71 mL/min/1.73m²), however the number of patients was small. Based on these results, it is not simple to answer the question from which age formulae developed for adults should be applied. The decision should be made for each patient individually and only for children who passed the teenage years. It is also advisable to estimate GFR by both formulae if the renal function has to be followed over a prolonged period of time.

COMPARISON OF TWO METHODS TO ESTIMATE THE GLOMERULAR FILTRATION RATE

In Chapter 2 - 5 several methods were compared to estimate GFR in children. Agreement between two methods was evaluated by calculating the predictive performance (bias and imprecision) and/or creating Bland-Altman plots. In the past, it was common to compute the correlation coefficient to evaluate the agreement between two measurement methods in the same subject. Sheiner et al. showed that a high correlation coefficient does not indicate an absence of over- or under prediction and can be quite misleading [19]. The correlation coefficient assesses the strength of the association along the best line between the two methods, but that line is not necessarily the line of identity, in which one is interested. If a correlation coefficient of 1 is found between an estimated and reference GFR and there is a systematic overprediction of 50 mL/min/1.73m² the method is still worthless. It is more accurate to evaluate the

predictive performance by calculating the mean relative prediction error (MPE) as a measure of bias and the root mean squared relative prediction error (RMSE) as measure of imprecision [19].

Bland and Altman have devised a simple way of graphically comparing two methods: the difference between two methods is plotted against the mean of the two methods [20]. The advantage of this method is that a trend in scatter can be observed easily. The mean difference between the two methods and its 95% confidence intervals can be calculated. The limits of agreement represent the range around the mean difference between the two methods in which 95% of the values will be found (mean difference ± 1.96 x SD). A small mean difference between two methods does not imply that they are interchangeable. If a mean difference of 3 mL/min/1.73m² is found between an estimated and reference GFR but the limits of agreement were 54 mL/min/1.73m² below and 47 mL/min/1.73m² above, the estimated GFR (usually a new method) would be unacceptable for clinical purposes, since one would not be able to detect small changes in GFR (for example 10 mL/min/1.73m²).

Comparing methods to estimate GFR in children, Bland-Altman plots are entering common use and the bias and imprecision have been calculated in several studies. It is interesting to speculate on the predictive performance if, for example, Schwartz et al. had used Bland-Altman plots in stead of the correlation coefficient in order to validate that formula.

In general, Bland-Altman plots should be created and/or the predictive performance (i.e. bias and imprecision) should be calculated, and it is out of date to plot the estimated value against the reference value, and state the correlation coefficient.

see pages 126 - 127 for references

CONCLUSION AND FINAL REMARKS

For many years it was common, when assessing renal function in children, to determine the inulin clearance (urinary or plasma clearance) by administering inulin by continuous intravenous infusion. Determination of the plasma clearance of inulin by the single injection method was less common, since for an accurate determination 10-12 blood samples were required. This number of blood samples is stressful and inconvenient for children. Recently, the number of blood samples required for the inulin single injection method has been reduced by the introduction of limited and optimal sampling strategies. The inulin single injection method with blood samples taken at 10, 30, 90 and 240 minutes after administration of inulin and calculation of GFR by Bayesian analysis with appropriate pharmacokinetic software like MW/Pharm, is accurate and convenient for children. This method is easy enough to apply in any hospital, but its utilization is limited since it is not very practical to use every day. For daily practice GFR in children can be estimated by using a formula based on the ratio of creatinine plasma concentration and body height, multiplied by a constant k, whose value is assessed locally. Based on our studies we recommend the formula with k = 40 (Morris et al.) as a good starting point.

REFERENCES

1. National Kidney Foundation (2002) K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification.
Am J Kidney Dis S1:S76-S110

2. European Best Practice Guidelines for Haemodialysis, Section I (2002) Nephrol Dial Transplan 17S7:7-15

3. Swinkels DW, Hendriks JCM, Nauta J, de Jong MCJW (2000) GFR by single-injection inulin clearance: definition of a workable protocol for children. Ann Clin Biochem 37:60-66

4. Van Rossum LK, Mathot RA, Cransberg K, Vulto AG (2003) Optimal sampling strategies to assess inulin clearance in children by the inulin single injection method.
Clin Chem 49:1170-1179

5. Van Rossum LK, Cransberg K, Mathot RAA, Vulto AG (2005) Bepaling van de glomerulaire filtratiesnelheid bij kinderen door middel van de inuline single injectie methode.
Tijdsch Kindergeneeskd 73:170-175

6. Van Rossum LK, Cransberg K, De Rijke YB, Zietse R, Lindemans J, Vulto AG (2005) Determination of inulin clearance by single injection or infusion in children.
Ped Nephrol 20:777-781

7. Cockroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31-41

8. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation.
Ann Intern Med 130:461-70

9. Levey AS, Greene T, Kusek JW, Beck GJ (2000) A simplified equation to predict glomerular filtration rate from serum creatinine.
J Am Soc Nephrol 11:A0828

10. Manjunath G, Sarnak MJ, Levey AS (2001) Prediction equations to estimate glomerular filtration rate: an update.
Curr Opin Nephrol Hypertens 10:785-92

11. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM (1976) Estimation of glomerular filtration rate from plasma creatinine concentration in children.
Arch Dis Child 51:875-878

12. Morris MC, Allanby CW, Toseland P, Haycock GB, Chantler C (1982) Evaluation of a height / plasma creatinine formula in the measurement of glomerular filtration rate.
Arch Dis Child 57:611-615

13. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259-263

14. Leger F, Bouissou F, Coulais Y, Tafani M, Chatelut E (2002) Estimation of glomerular filtration rate in children. Pediatr Nephrol 17:903-907

15. Van Rossum LK, Mathot RA, Cransberg K, Zietse R, Vulto AG (2005) Estimation of the glomerular filtration rate in children: which algorithm should be used? Ped Nephrol 20:1769-1775

16. Pierrat A, Gravier E, Saunders C, Caira MV, Ait-Djafer Z, Legras B, Mallie JP (2003) Predicting GFR in children and adults: a comparison of the Cockcroft-Gault, Schwartz, and modification of diet in renal disease formulae. Kidney Int 64:1425-36

17. Paap CM, Nahata MC (1995) Prospective evaluation of ten methods for estimating creatinine clearance in children with varying degrees of renal dysfunction. J Clin Pharm Ther 20:67-73

18. Filler G, Foster J, Acker A, Lepage N, Akbari A, Ehrich JH (2005) The Cockcroft-Gault formula should not be used in children. Kidney Int 67:2321-4

19. Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokin Biopharm 9:503-51

20. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307-310

CHAPTER 8

SUMMARY

Renal function tests are important to evaluate the progression of kidney disease and to monitor renal function during drug treatment. For the assessment of renal function the mechanisms responsible for the formation of urine, namely filtration, reabsorption and secretion, must be taken into consideration. The excretion of endogenous and exogenous substances is mainly regulated by glomerular filtration. Glomerular filtration rate (GFR) is considered the most important parameter to evaluate renal function and it is affected by most kidney diseases.

For children's convenience it is important that the determination of GFR can be performed in a short time window with minimal intervention. On the other hand it is important that the test shows a high accuracy. In general, tests that are most accurate are also those that are most elaborate and costly.

The aim of this thesis was to develop a simple, practical, convenient and accurate method to determine the glomerular filtration rate adapted to the specific requirements of children.

In Chapter 1 the advantages and disadvantages of several methods to determine GFR in children for daily practice and in a clinical research setting are described. Also, the practical aspects of endogenous (creatinine and cystatin C) and exogenous (inulin, radio-isotopes and iohexol) markers are discussed.

In Chapter 2 we describe the development and the validation of several sampling strategies with a reduced number of samples to predict the inulin plasma clearance in pediatric patients using the inulin single injection method. First a population pharmacokinetic model for inulin was developed for the index data set (n=100) and included the variables BSA (body surface area) and HUS (hemolytic uremic syndrome). Then optimal sampling times were selected and validated in the validation data set (n=54). Strategies with 2 to 4 samples, which should include a sample at 240 min after administration of inulin, produced an accurate prediction of inulin clearance in the validation

data set (bias < 3% and not significantly different from zero, imprecision < 15%). Even 1 blood sample at 240 min showed an acceptable performance, however there is no room for errors (pre-analytical or analytical errors). The proposed strategies with 2 - 4 blood samples (10 / 30 / 90 / 240 min, 10 / 30 / 240 min, 10 / 90 / 240 min, 30 / 90 / 240 min and 90 / 240 min) are practical and convenient to children and can be applied to determine the inulin plasma clearance in children.

In Chapter 3 the inulin plasma clearance determined by the single injection method is compared with the reference method (continuous infusion method) in 24 pediatric patients. The inulin plasma clearance determined by the single injection method with 4 blood samples was on average 9.7 mL/min/1.73m² higher than the clearance determined with the continuous infusion method (95% CI: 5.3; 14.2). The difference between both methods was smaller at lower GFRs and it was considered acceptable for clinical practice. As a consequence the determination of the inulin plasma clearance with continuous infusion, which was applied as the standard method to determine GFR in children for many years in the Sophia Children's Hospital, can be replaced by the inulin single injection method. The last method is less time-consuming and more convenient since no overnight hospitalization is required.

In Chapter 4 the validation of the inulin single injection method with a limited number of blood samples (10 / 30 / 90 / 240 min) in 48 pediatric patients using the pharmacokinetic program MW/Pharm is reported. In patients with GFR < 75 mL/min/1.73m² the mean difference between the inulin plasma clearance based on 4 blood samples and the reference clearance (based on 11 blood samples) was not statistically significant. For GFR > 75 mL/min/1.73m² a small bias was observed (mean difference: -5.92 mL/min/1.73m²; 95% confidence interval: -10.2; -1.68 mL/min/1.73m²). The pharmacokinetic program MW/Pharm, which is available in nearly all hospital pharmacies, can be used to calculate the inulin plasma clearance in children determined by the single injection method with 4 blood samples. In this way, the optimized method can easily be implemented in every Dutch hospital.

For daily practice the glomerular filtration rate in children can be estimated by GFR = k x BH / Pcr (BH: body height (cm); Pcr: plasma creatinine concentration (µmol/L)). In Chapter 5 the predictive performance of formulae to estimate GFR in children is evaluated. In 48 children (aged from 5 - 18 years; GFR: 7 - 158 ml/min/1.73m²) GFR

estimated by formulae was compared with the inulin plasma clearance. The formula with a k value of 41.2 or 40 showed the best predictive performance. Introduction of more patient factors (age, body surface area, body weight and gender) resulted in an algorithm, that did not improve the predictive performance. However, the performance of the formulae is closely linked with the method, which was used for an accurate determination of GFR (reference method) and the population of patients. As a consequence it is recommended to assess the optimal value for k locally.

In Chapter 6 the use of cystatin C as an endogenous marker for GFR is described. Cystatin C is a relatively new marker for GFR and has been suggested as an alternative for creatinine. We applied the renal extraction ratio of cystatin C to evaluate whether cystatin C is useful to estimate GFR. The renal extraction ratio is a parameter for renal function and expresses the glomerular filtration and tubular handling (tubular secretion and / or reabsorption) of a substance. The renal extraction of cystatin C was compared with that of ^{125}I-iothalamate in 40 hypertensive adult patients. The mean difference between the renal extraction ratio of cystatin C and that of ^{125}I-iothalamate was 0.002 (95% confidence interval :-0.036 to 0.032). However, the limits of agreement were large (-0.271 and 0.267) and not acceptable. Therefore, the use of cystatin C to estimate GFR is limited and as a consequence it cannot replace the traditional endogenous marker creatinine.

In conclusion, the inulin single injection method with blood samples taken at 10, 30, 90 and 240 minutes after administration of inulin and calculation of GFR by Bayesian analysis with appropriate pharmacokinetic software like MW/Pharm, is an accurate method to determine GFR in children. This method is also convenient for children and easy enough to apply in any hospital. For daily practice GFR in children can be estimated by using a formula based on the ratio of creatinine plasma concentration and body height, multiplied by a constant k, whose value is assessed locally. The formula with k = 40 (Morris et al.) is a good starting point to estimate GFR in children.

This thesis describes how the method for the determination of GFR in childeren is simplified and is an exellent example how to solve an important clinical problem in a multi-disciplinary fashion.

SAMENVATTING

Het testen van de nierfunctie is belangrijk voor het evalueren van de voortgang van een nierziekte en het volgen van de nierfunctie tijdens behandeling met geneesmiddelen. Voor het bepalen van de nierfunctie kunnen verschillende stoffen (markers) worden gebruikt. Markers voor de nierfunctie kunnen worden onderverdeeld in lichaamseigen en niet-lichaamseigen stoffen. Inuline, een groot suikermolecuul, wordt gezien als ideale marker. Het behoort tot de niet-lichaamseigen markers. Kreatinine en cystatine C zijn voorbeelden van lichaamseigen markers. Kreatinine wordt in de dagelijkse praktijk gebruikt voor het schatten van de nierfunctie op basis van een formule. Voor het nauwkeurig bepalen van de nierfunctie, zoals gewenst is in bijvoorbeeld een onderzoeksomgeving, is deze methode niet geschikt. De klassieke methode voor het nauwkeurig bepalen van de nierfunctie is gebaseerd op het toedienen van een bekende dosis inuline en het verschijnen van inuline in de urine. Bij de alternatieve methode wordt na toediening het verdwijnen van inuline uit het bloed gevolgd. Het is gebruikelijk om de nierfunctie bij kinderen gestandaardiseerd uit te drukken in ml/min/1.73m^2 lichaamsoppervlakte, zodat verschillen tussen kleine en grote kinderen worden opgeheven.
Voor het welzijn van kinderen is het belangrijk dat de test voor het bepalen van de nierfunctie kan worden uitgevoerd in een kort tijdsbestek met minimale belasting.
Aan de andere kant is het belangrijk dat de test een hoge nauwkeurigheid heeft. In het algemeen zijn de meer nauwkeurige testen minder patiëntvriendelijk.
Het doel van dit proefschrift is het ontwikkelen van een eenvoudige, praktische, patiëntvriendelijke en nauwkeurige methode voor het bepalen van de nierfunctie bij kinderen.

In Hoofdstuk 1 worden de voor- en nadelen van verschillende methoden voor het bepalen van de nierfunctie bij kinderen beschreven, zowel voor de dagelijkse klinische praktijk als voor een onderzoeksomgeving. Eveneens worden de praktische aspecten van

lichaamseigen (kreatinine en cystatine C) en niet-lichaamseigen (inuline, radio-isotopen en iohexol) markers voor het bepalen van de nierfunctie bediscussieerd.

In Hoofdstuk 2 wordt het opstellen en testen van diverse bemonsteringsschema's voor het bepalen van de nierfunctie bij kinderen beschreven. Hierbij werd uitgegaan van de inuline eenmalige injectie methode. Inuline werd als eenmalige gift via de bloedbaan gegeven en twaalf bloedmonsters werden in een tijdsbestek van 240 minuten afgenomen. Op grond van de inuline concentratie in de afgenomen bloedmonsters, werd de nierfunctie berekend. Het doel van dit onderzoek was om het aantal af te nemen bloedmonsters te verminderen. Hiervoor werd een populatie farmacokinetisch model voor inuline ontwikkeld en werden optimale bemonsteringstijdstippen vastgesteld. Vervolgens werden diverse bemonsteringsschema's getest in een data set met gegevens van 54 kinderen. Bemonsteringsschema's met 2 tot 4 bloedmonsters, inclusief een bloedmonster 240 minuten na toedienen van inuline, leverde een nauwkeurige voorspelling van de nierfunctie op. Zelfs 1 bloedmonster op 240 min leverde een acceptabel resultaat op, maar het gebruik van 1 bloedmonster is erg risicovol.
De bemonsteringsschema's met 2 - 4 bloedmonsters (10 / 30 / 90 / 240 min, 10 / 30 / 240 min, 10 / 90 / 240 min, 30 / 90 / 240 min, en 90 / 240 min) zijn praktisch en kindvriendelijk, leveren een nauwkeurig resultaat op en kunnen goed worden gebruikt voor het bepalen van de nierfunctie bij kinderen.

In Hoofdstuk 3 wordt de nierfunctie bepaald met de inuline eenmalige injectie methode, vergeleken met de continue infusie methode. De continue infusie methode was lange tijd de standaard methode voor het bepalen van de nierfunctie bij kinderen in het Sophia Kinderziekenhuis. De methode bestaat uit het toedienen van inuline via de bloedbaan tot een constante concentratie inuline in het bloed is bereikt. Voor deze methode moet de patiënt een nacht worden opgenomen, terwijl de eenmalige injectie methode in 4 uur kan worden uitgevoerd. Bij 24 kinderen werd de inuline eenmalige injectie methode vergeleken met de continue infusiemethode. De nierfunctie bepaald met de eenmalige injectie methode met 4 bloedmonsters (10 / 30 / 90 / 240 min) was gemiddeld 9.7 mL/min/1.73m² hoger dan de nierfunctie bepaald met de continue infusie methode. Het verschil tussen beide methoden was kleiner bij slechtere nierfunctie en was acceptabel in relatie tot de klinische praktijk. Op basis van deze resultaten kan de continue infusie methode worden vervangen door de inuline eenmalige injectie methode. Deze laatste methode is

kindvriendelijker en kost minder tijd, omdat geen opname gedurende de nacht is vereist.

In Hoofdstuk 4 wordt het testen van de inuline eenmalige injectie methode met een verminderd aantal bloedmonsters en gebruik makend van het computerprogramma MW/Pharm beschreven. Voor het berekenen van de nierfunctie aan de hand van de inuline eenmalige injectie methode met een verminderd aantal bloedmonsters is een computerprogramma nodig, dat gebruik maakt van het populatie farmacokinetisch model van inuline. Het computerprogramma MW/Pharm, dat beschikbaar is in bijna elke ziekenhuisapotheek, werd hiervoor gebruikt. Voor 48 kinderen werd de nierfunctie op basis van 4 bloedmonsters (10 / 30 / 90 / 240 min) berekend met het computerprogramma MW/Pharm en vergeleken met de nierfunctie gebaseerd op alle bloedmonsters (referentie nierfunctie). Bij kinderen met een slechtere nierfunctie was er geen verschil tussen de berekende nierfunctie met MW/Pharm en de referentie nierfunctie. Bij kinderen met een matige tot normale nierfunctie werd een klein maar acceptabel verschil waargenomen (gemiddeld verschil : -5.92 mL/min/1.73m²). Op grond van deze resultaten kan het computerprogramma MW/Pharm worden gebruikt voor het berekenen van de nierfunctie bij kinderen bepaald met de inuline eenmalige injectie methode met 4 bloedmonsters. Met deze gegevens kan de geoptimaliseerde inuline eenmalige injectie methode eenvoudig worden toegepast in elk Nederlands ziekenhuis.

In Hoofdstuk 5 wordt het gebruik van verschillende formules voor het schatten van de nierfunctie bij kinderen beschreven. Voor de dagelijkse praktijk is het bepalen van de nierfunctie met de inuline eenmalige injectie methode niet praktisch en wordt de nierfunctie bij kinderen geschat aan de hand van een formule, waarbij gebruik gemaakt wordt van de lichaamseigen marker kreatinine. De formule is gebaseerd op de lichaamslengte van het kind, de concentratie kreatinine in het bloed en een constante:

$$\text{Nierfunctie} = \frac{\text{lichaamslengte x constante}}{\text{concentratie kreatinine}}$$

Voor de constante zijn in de literatuur verschillende waardes beschreven. In dit onderzoek werd bij 48 kinderen de nierfunctie geschat aan de hand van diverse formules en vergeleken met de nierfunctie gebaseerd op de inuline eenmalige injectie methode. De formule met een constante van 41.2 of 40 leverde het beste resultaat op. Introductie van meer patiënt factoren naast lichaamslengte (leeftijd, lichaamsoppervlakte, lichaamsgewicht en geslacht) leidde niet tot een beter resultaat.
Het gebruik van de formules hangt nauw samen met de methode, die lokaal werd gebruikt voor het bepalen van de nierfunctie, en met de patiëntengroep. Om deze reden is het aan te bevelen om lokaal de optimale waarde voor de constante te bepalen.

In Hoofdstuk 6 wordt het gebruik van cystatine C als lichaamseigen marker voor de nierfunctie beschreven. Cystatine C is een relatief nieuwe marker en mogelijk een alternatief voor kreatinine. In dit onderzoek werd de renale extractie ratio van cystatine C bepaald om te beoordelen of cystatine C geschikt is voor het schatten van de nierfunctie. De renale extractie ratio van cystatine C werd vergeleken met de renale extractie ratio van ^{125}I-iothalamaat (niet-lichaamseigen marker) in 40 volwassenen met een hoge bloeddruk. Het gemiddeld verschil tussen de renale extractie ratio van cystatine C en die van ^{125}I-iothalamaat was klein. Echter, de spreiding was erg groot en niet acceptabel. Om deze reden is cystatine C voor het schatten van de nierfunctie van beperkte waarde en kan het de traditionele marker kreatinine niet vervangen.

Terugkomend op het doel van dit proefschrift, zoals beschreven staat in het begin van deze samenvatting, kan worden geconcludeerd dat de inuline eenmalige injectie methode met bloedmonsters afgenomen op 10, 30, 90 en 240 minuten na toediening van inuline en berekening van de nierfunctie met het computerprogramma MW/Pharm, een geschikte methode is om de nierfunctie bij kinderen te bepalen. Deze methode is eveneens kindvriendelijk en makkelijk toepasbaar in elk ziekenhuis.
Voor het schatten van de nierfunctie bij kinderen in de dagelijkse praktijk kan gebruik worden gemaakt van een formule gebaseerd op lichaamslengte, kreatinine concentratie in het bloed en een constante, die lokaal is vastgesteld. De formule met als constante $k = 40$ is een goed startpunt voor het schatten van de nierfunctie bij kinderen.
Tot slot, kan worden opgemerkt dat met dit onderzoek de methode voor het nauwkeurig bepalen van de nierfunctie bij kinderen sterk is vereenvoudigd, wat zowel voor het kind als voor degene die de test uitvoert voordelen heeft. Tevens is het onderzoeksproject een mooi voorbeeld van hoe een belangrijk klinisch probleem met een multidisciplinaire aanpak kan worden opgelost.

LIST OF PUBLICATIONS

FULL PAPERS

- Van Rossum LK, Mathot RAA, Cransberg K, Vulto AG (2003) Optimal sampling strategies to assess inulin clearance in children by the inulin single-injection method. Clin Chem 49:1170-1179

- Van Rossum LK, Cransberg K, De Rijke YB, Zietse R, Lindemans J, Vulto AG (2005) Determination of inulin clearance by single injection or infusion in children. Pediatr Nephrol 20:777-781

- Van Rossum LK, Cransberg K, Mathot RAA, Vulto AG (2005) Bepaling van de glomerulaire filtratiesnelheid bij kinderen door middel van de inuline single injectie methode. Tijdschr Kindergeneeskd 73:170-175

- Van Rossum, Mathot RAA, Cransberg K, Zietse R, Vulto AG (2005) Estimation of the glomerular filtration rate in children: which algorithm should be used? Pediatr Nephro 20:1769-1775

- Van Rossum LK, Zietse R, Vulto AG, De Rijke YB. Renal extraction of cystatin C versus ^{125}I-iothalamate in hypertensive patients. Submitted for publication

- Van Rossum LK (2004) Doseren van genees-middelen bij verminderde nierfunctie. In: BH Graatsma en R de Jong (red). Geneeskundig Jaarboek blz 23-29

- Kist-van Holthe JE, LK van Rossum (2002) Dosering van geneesmiddelen bij verminderde nierfunctie. In: AJ van der Heijden, CH Schröder en JAE van Wijk (red). Werkboek kindernefrologie blz 251-266

ABSTRACTS

- Van Rossum LK, Mathot RAA, Cransberg K, Vulto AG (2003) Determination of glomerular filtration rate in children by the inulin single injection method. Ped Transplantation 7(4S):105

- Van Rossum LK, Cransberg K, Mathot RAA, Vulto AG (2003) Estimation of AUC for monitoring of cyclosporine in pediatric patients. Ped Transplant 7(4S):102

- Van Rossum LK, Cransberg K, Mathot RAA, Weterings S, Vulto AG (2003) AUC estimation for routine therapeutic drug monitoring of cyclosporine in pediatric patients. Clin Pharmacol Ther 2:P88

- Van Rossum LK, Mathot RAA, Vulto AG (2002) Optimal sampling strategies for the assessment of inulin clearance in children. Clin Pharmacol Ther 71:P80

- Van Rossum LK, De Rijke YB, Derkx F, Lindemans J, Vulto AG (2002) Assessment of renal extraction ratio of cystatin C in hypertensive patients. Clin Pharmacol Ther 71:P46

- Van Rossum LK, Mathot RAA, Cransberg K, Vulto AG (2001) Een optimaal bemonsteringsschema voor het bepalen van de glomerulaire filtratiesnelheid (GFR) bij kinderen gebaseerd op de inuline single-injection methode. Tijdschr Kindergeneeskd 69(5A):4

- Van Rossum LK, Cransberg K, Derkx F, Lindemans J, De Rijke YB, Zietse R and Vulto AG (2001) The result of determination of the GFR in children by the inulin single-injection-method is computer software dependent. Br J Clin Pharm 51:493

DANKWOORD

Het doen van onderzoek gaat altijd gepaard met mooie momenten (de laatste patiënt is geïncludeerd, een artikel is geaccepteerd) en tegenslagen (product is tijdelijk niet leverbaar). Juist die afwisseling, waarbij uiteindelijk de mooie momenten overheersen, maakt het doen van onderzoek zeer interessant. Een ander aantrekkelijk aspect is het multidisciplinaire karakter van onderzoek. Mede-onderzoekers kijken vaak net iets anders tegen een probleem aan en leveren een belangrijke bijdrage aan het welslagen van het onderzoek. Daarom wil ik de volgende mensen bedanken.

Allereerst mijn promotor Prof. dr. A.G. Vulto, die mij enthousiast heeft gemaakt voor het doen van onderzoek. Beste Arnold, door jouw enthousiasme en begeleiding kan het ZAPIKO-project succesvol worden afgerond. Verder heb ik veel geleerd van jouw heldere analyses en dank ik je voor de prettige samenwerking.
Daarnaast wil ik de mede-onderzoekers drs. K. Cransberg, Prof. dr. J. Lindemans, dr. Y.B. de Rijke en dr. R. Zietse bedanken voor hun betrokkenheid bij het onderzoek. Beste Karlien, bedankt voor de zeer prettige samenwerking, zonder jou had het includeren van patiënten oneindig veel langer geduurd. En ik waardeer het dat je mijn paranimf wilt zijn. Beste Jan, veel dank voor jouw gastvrijheid en voor de zeer waardevolle opmerkingen, waarmee je het onderzoek diepte gaf. Beste Yolanda, ontelbare metingen van inuline en cystatine C zijn onder jouw begeleiding uitgevoerd. Ik wil je daarvoor en voor de prettige samenwerking bedanken. Beste Bob, jouw bijdrage aan het onderzoek als nefroloog / clinicus heb ik altijd zeer op prijs gesteld. Ik heb veel geleerd van de discussies die we hebben gevoerd en van jouw ervaring als onderzoeker.
Eveneens wil ik dr. R.A.A. Mathôt bedanken voor zijn bijdrage aan het onderzoek. Beste Ron, je bent pas in een later stadium bij het onderzoek betrokken geraakt (in eerste instantie op afstand en later op locatie), maar je hebt me op jouw eigen wijze wegwijs gemaakt in de wonderlijke wereld van de farmacokinetiek.

Dr. F. Derkx wil ik ook bedanken voor zijn betrokkenheid bij het onderzoek. Beste Frans, jammer dat je niet tot het einde van het cystatine C onderzoek erbij kon zijn.

De leden van de kleine promotiecommissie, Prof. dr. A.J. van der Heijden, Prof. dr. J. Lindemans en Prof. dr. L. Monnens, wil ik bedanken voor het beoordelen van het manuscript en voor hun bereidheid deel te nemen aan deze promotie.

De verpleegkundigen van de thuisdialyse-Sophia, Caro Fonkert, Marja Kenselaar, Katinka van Linschoten, Corin Verburg en Marjoleine van der Zijde, dank ik zeer voor het uitvoeren van de vele inuline klaringstesten.
De analisten van de afdeling Klinische Chemie-Sophia, Barrie Koelewijn and Sacha Smit, wil ik bedanken voor hun inzet bij het uitvoeren van zowel de inuline als cystatine C bepalingen. Reinier van Hest, destijds stagiaire en nu mijn ZAPIKO-collega, wil ik bedanken, voor het opzetten van de cystatine C bepaling.
Eveneens wil ik dr. D.W. Swinkels, dr. J.C.M. Hendriks en dr. J. Nauta bedanken voor het beschikbaar stellen van de data-set en voor de interessante discussies over populatiekinetiek.

Verder wil ik mijn collega ziekenhuisapothekers in opleiding, de ziekenhuis-apothekers, projectapothekers, apothekersassistenten, analisten en administratief, farmaceutisch, logistiek, secretarieel en andere medewerkers van de apotheek bedanken voor hun belangstelling voor en betrokkenheid bij mijn onderzoek.

Caroline Couwenbergh en Martijn Bertram van Design onderweg wil ik hartelijk bedanken voor het vormgeven van het proefschrift. Ik ben erg tevreden over het eindresultaat.

Mijn vader en Marjo, Michelle en René, en mijn vrienden wil ik bedanken omdat ze mijn onderzoek met veel belangstelling hebben gevolgd en omdat ik altijd bij hen terecht kan, ongeacht of het leuke of minder leuke dingen betreft. Lieve papa en Michelle, jullie wil ik nog in het bijzonder bedanken omdat ik het zonder jullie steun nooit zover zou hebben gebracht.