Estimation of renal function

How good are clinical measures of glomerular filtration rate?

Henry Quiñones, M.D.

INTERNAL MEDICINE GRAND ROUNDS
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS

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The kidney has many functions (Fig 1) (1). Some of them are control of vitamin D production, erythropoietin production, acid-base balance, free water homeostasis, regulation of the renin-angiotensin system, solute balance, excretion of uremic toxins, filtration of plasma, and many others (5, 6, 7). As the kidney function goes down, all of these diverse functions of the kidney go down, too. Vitamin D production decreases, erythropoietin production decreases leading to decreases in hematocrit, acid excretion decreases leading to metabolic acidosis, the capability of maximum urine osmolality and minimum urine osmolality decreases and approaches plasma osmolality, the renin-angiotensin system gets activated, the body starts getting on positive balance of solutes like potassium, and the excretion of uremic toxins is impaired (see Table 1) (2, 3, 7). However, if we are to use some or all of these parameters as markers of general renal function, the renal function needs to be decreased by 50% or more before we can start observing anyone of these abnormalities. Moreover, some of these parameters can be affected by other physiologic or pathophysiologic processes in the body. For example, the hematocrit can decrease because of gastrointestinal bleeding, iron deficiency, hemolysis, etc., and not necessarily

Fig. 1 **Functions of the kidney**

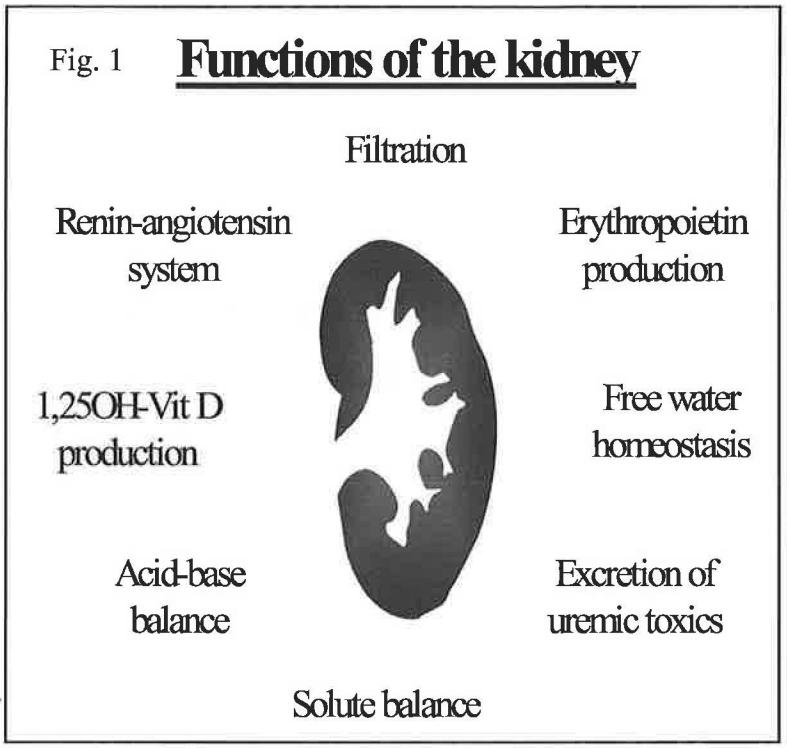


Table 1 Parameters to follow with decreased renal function

GFR (% of 180L/day)	100	65	33	20	10
P_{HCO3} (mEq/L)	24	24	22	16	13
Hematocrit (%)	42	40	38	32	24
P_{cr} (mg/dL)	1	1.6	3.1	5.0	10.4
P_{BUN} (mg/dL)	14	18	29	46	82
P_{pH}	7.4	7.4	7.37	7.3	7.26
Max U_{osm} (mOsm/kg)	1200	1000	500	350	310
Min U_{osm} (mOsm/kg)	50	50	70	200	310

due to a decrease in renal function (see Table 1). For these reasons it is not practical to use them as accurate markers of renal function, and we end using glomerular filtration as a very specific parameter to follow general renal function.

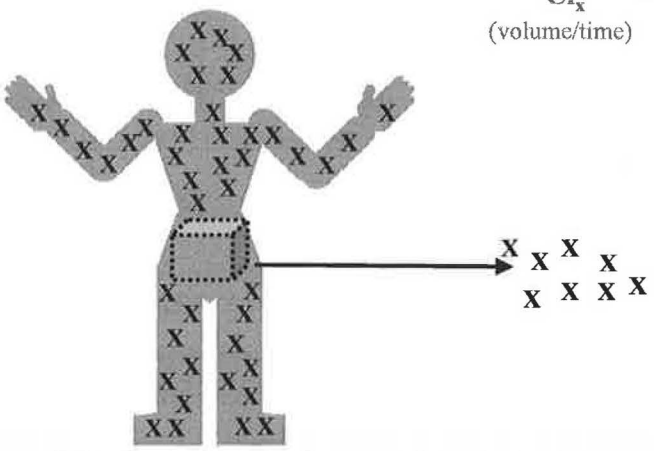
Fig. 2 **Concept of clearance**

$$Cl_x \times [x] = Excretion_x$$

(volume/time) (mass/volume) (mass/time)

$$Cl_x = \frac{Excretion_x}{[x]}$$

(volume/time) (mass/time) (mass/volume)



The more frequent method that we use to estimate glomerular filtration rate is clearance. The concept of clearance starts with the statement that excretion of a substance x from the body in a determined period of time, is equal to the concentration of x in the body, multiplied by the volume of the body that gets completely cleared of x in a determined period of time. As you can realize, clearance of x (Cl_x) is a virtual volume of the body that is getting cleared completely of a substance x over a specific period of time (8, 9). If this concept is true, then you can resolve the equation for clearance of x. Clearance of x will be equal to the excretion of x over a determined period of time, divided by the concentration of x in the body (see Fig.2).

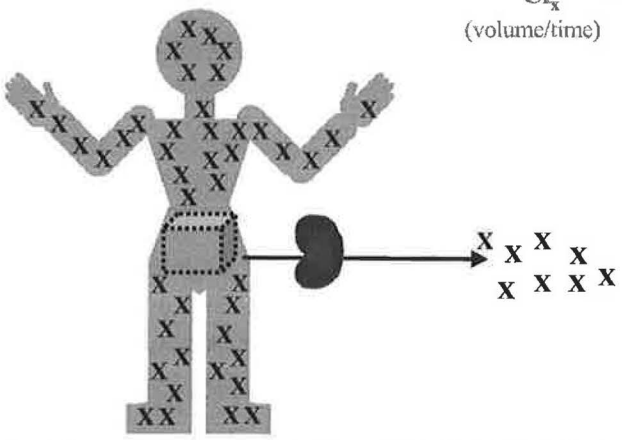
Fig. 3 **Concept of clearance**

$$Cl_x \times P_x = U_x \times V$$

(volume/time) (mass/volume) (mass/time)

$$Cl_x = \frac{U_x \times V}{P_x}$$

(volume/time) (mass/time) (mass/volume)

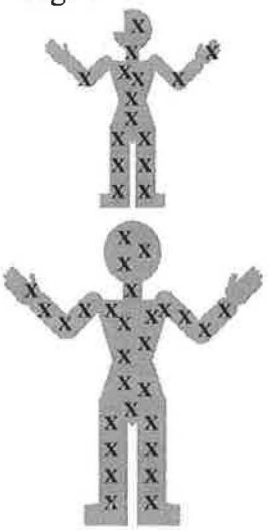


If we then focus on renal clearance, then excretion of x will become urinary excretion of a substance x (e.g., $U_x \times V$), the concentration of x will become the plasma concentration of x (e.g., P_x) as this is the volume that the kidney is clearing from, and clearance will become the volume of plasma that is getting completely

cleared of x over a determined period of time (see Fig. 3). The concept of clearance could then be a good approximation of glomerular filtration rate (GFR). However, the kidney can filter substance x and after filtering it, some of substance x can be secreted or reabsorbed. If the kidney filters and secretes substance x, $U_x \times V$ will be more than what it would have been if only filtration would have occurred. In this situation Cl_x will overestimate GFR. On the other hand, if the kidney filters and reabsorbs substance x, $U_x \times V$ is going to be less than what it would have been if only filtration would have occurred. Now Cl_x will be an underestimation of GFR. Only if the kidney filters substance x, then Cl_x will be equal to GFR.

Fig. 4

Concept of clearance



Cl_x	[x]	$Excretion_x$	$Production_x$
100	1.0	100	100
50	2.0	100	100
100	1.5	150	150
50	3.0	150	150

Another way of looking at the concept of clearance is revealed by resolving the equation for concentration of x. The concentration of x is inversely related to clearance of x and directly related to excretion of x. Excretion of x is equal to production of x in a steady state situation. In other words, the lower the clearance of x, the higher the concentration of x will be when a new steady state is achieved, and vice versa. Also, if the clearance is constant, then concentration of x will be determined by excretion of x, which in steady state is equal to production of x. Therefore, consider two hypothetical situations of two individuals (see Fig. 4), one small size and the other large sized, both with the same clearance of x of 100 volume/time. The production of x in the small individual is 100 mass/time which is equal to excretion of 100 mass/time in steady state. Because concentration of x is equal to excretion of x divided by clearance of x, then you can calculate that concentration of x is 1mass/vol. If the clearance goes down to 50 volume/time, then after going through the same calculation, the new concentration of x will be 2 mass/volume once the new steady state is achieved. Now, the large individual has a production of x of 150 mass/time that equals excretion of x of 150.mass/time in steady state. Using the same equation we can calculate the concentration of x to be 1.5 mass/volume compared to 1.0 mass/volume in the small individual. Similarly, if the clearance of x decreases to 50 volume/time, then the concentration of x will go up to 3.0 mass/volume, compared to 2.0 mass/volume in the smaller individual. Consequently, we can conclude that clearance of x is a better way of approximating GFR than the concentration of x as, for equal levels of clearance, the concentration of x can be very different in different individuals.

The concept of clearance is also valid in the setting of non-steady state (see Fig. 5), for example in the situation of acute renal failure. If the small individual of the previous example had a decrease in clearance of x from 100 volume/time to 10 volume/time and does not recover, the excretion of x will also go from 100 mass/time to 10 mass/time. However, now, the production is 100 mass/time and the excretion is 10 mass/ time which will cause the concentration of x to rise slowly. First the concentration of x will go up to 2 mass/volume but considering that clearance is 10 volume/time, the excretion goes up to 20 mass/time. This slow increase in concentration of x will continue to happen as long as production exceeds excretion and will stop when production equals excretion of x at a new steady state. In this case, that will happen when concentration of x will get to be 10 mass/volume. Going back to the equation, excretion is equal to concentration of x multiplied by clearance of x, in this case 10 volume/time multiplied by 10 mass/volume equals excretion of x of 100 mass/time which is as much as production of x in this individual. This also confirms our previous conclusion that clearance of x is a better estimate of GFR as in this example, we also see that for the same level of clearance of x of 10 the concentration of x is very variable in the non-steady state.

Fig. 5 **Concept of clearance**

GFR	Cl_x	[x]	$Excretion_x$	Production
100	100	1	100	100
10	10	1	10	100
10	10	2	20	100
10	10	3	30	100
10	10	4	40	100
10	10	5	50	100
10	10	6	60	100
10	10	7	70	100
10	10	8	80	100
10	10	9	90	100
10	10	10	100	100

Fig. 6 **If no reabsorption or secretion,**

$$GFR \times [P]_x = [U]_x \times V$$

$$GFR = \frac{[U]_x \times V}{[P]_x}$$

GFR = Clearance of x

If X is secreted,

$$GFR \times [P]_x < [U]_x \times V$$

$$GFR < \frac{[U]_x \times V}{[P]_x}$$

$$GFR < Cl_x$$

If X is reabsorbed,

$$GFR \times [P]_x > [U]_x \times V$$

$$GFR > \frac{[U]_x \times V}{[P]_x}$$

$$GFR > Cl_x$$

Lets come back to the renal clearance concept. As illustrated in Fig. 6, if x is not secreted nor reabsorbed, then urinary excretion of x ($U_x \times V$) is going to be equal to glomerular filtration of x ($GFR \times P_x$). Resolving the equation for GFR will lead to the conclusion that Cl_x equals GFR. The situation is different when x is filtered and secreted because now, urinary excretion of x ($U_x \times V$) is

going to be larger than glomerular filtration of x ($GFR \times P_x$), consequently, Cl_x is going to be an overestimation of GFR. Finally, if substance x is filtered and reabsorbed by the kidney, then the excretion of x ($U_x \times V$) will be less than the glomerular filtration of x ($GFR \times P_x$), and Cl_x will be an underestimation of GFR.

So how close we'll be to GFR using the concept of renal clearance is going to depend on what we select to represent x as a marker of GFR. Therefore lets revise what are the characteristics of an ideal marker of GFR. An ideal marker of GFR should not bound to proteins, should get freely filtered in the glomerulus, should not be reabsorbed or secreted by renal tubules, should not have any effect on renal function, should not be metabolized, and should not be extrarenally eliminated. After reviewing these, two groups of possible GFR markers come in to mind: endogenous GFR markers and exogenous GFR markers (see Fig. 7). The endogenous GFR markers are produced at a constant rate by our metabolism, are added to plasma in a constant fashion and are easy to measure. The exogenous markers of GFR are substances that we can inject into plasma at a constant rate, not produced by our own metabolism and the protocols for their

Fig. 7

GFR Markers:

- Endogenous:
  - Creatinine
  - Urea
  - Cystatin C
- Exogenous:
  - Inulin
  - Iohexol
  - Chromium-51-EDTA
  - Technetium-99m-DTPA
  - Iodine-125-iothalamate

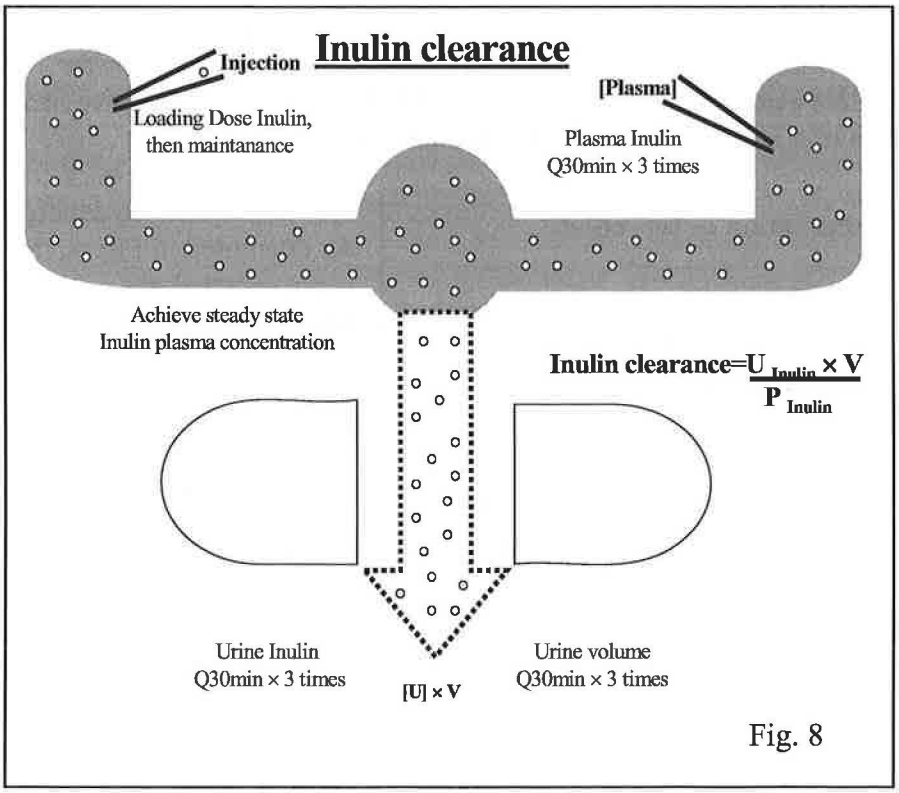


Fig. 8

measurement are time consuming. The list of endogenous and exogenous GFR markers is shown in Fig. 7.

The gold standard test to approximate GFR is measurement of inulin clearance. Inulin is a 5200-Da uncharged polymer of fructose that satisfies the criteria for an ideal GFR marker since it is freely filtered at the glomerulus, and it is not reabsorbed, secreted, or metabolized by the renal tubules. But, as well as the practical disadvantages of a continuous infusion, the anthrone method, often used to assay inulin, is complicated. For these reasons this assay is not accessible to common clinical practice. The most important steps of this procedure are shown in Fig. 8, and they evidence the impracticality of this procedure in most clinical scenarios.

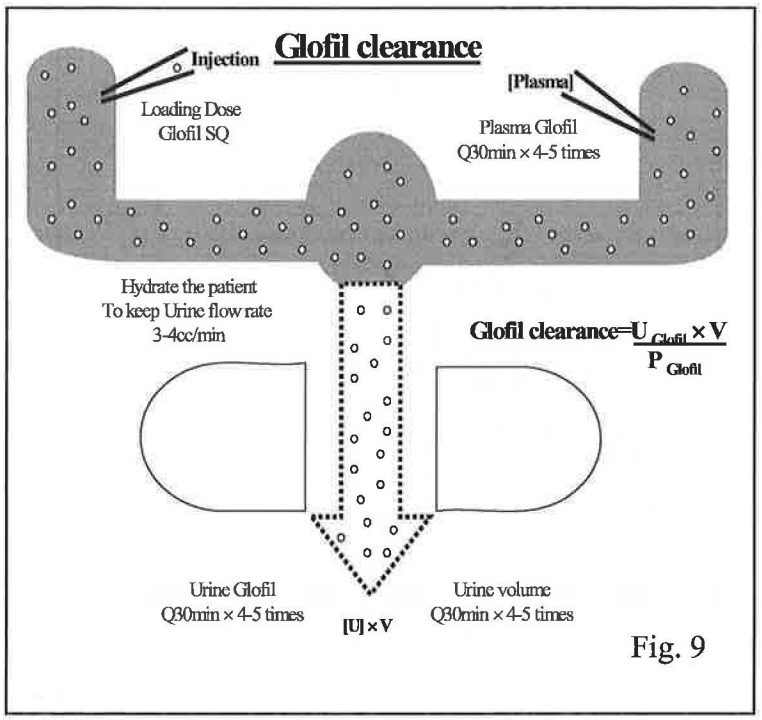


Fig. 9

The next most used technique to approximate GFR is ^{121}I-iothalamate clearance (Glofil clearance) (shown in Fig. 9) (12). The procedure requires a subcutaneous injection of glofil 30min after an IV injection of a saturated solution of potassium iodine to inhibit thyroid iodine uptake. After the glofil is injected the patients are hydrated to keep a urine flow of 3-4 mL/min. Plasma and urine samples are obtained every 30 min since the injection of glofil and the urine volume is charted simultaneously for each series of samples. This is repeated 4-5 times. Glofil is measured by means of a radioisotope scintillation counter which is easier than the anthrone method to measure inulin. GFR is calculated using the clearance formula. The problem with this technique is that the procedure is still somewhat laborious and that this is mostly available in big academic and biomedical research centers but it is still not widely available for clinical common practice.

We find ourselves then mostly using creatinine clearance as an estimate of GFR. Creatinine is filtered by the glomerulus and secreted by the proximal tubule of the kidney. As explained before, creatinine clearance will be an overestimation of GFR. This is confirmed by Fig. 10 is showing by means of this plot of the measured creatinine clearance against ^{121}I-iothalamate clearance (18). From this plot we can make a few observations. The higher the GFR the more scattered the data points are in relation to the identity line. The lower the GFR, the closer the data points are to each other. A few data points approaches the identity line suggesting that creatinine clearance can occasionally approach GFR Other data points shows that creatinine clearance can underestimate

GFR. However, most of the time measured creatinine clearance is an overestimation of the GFR.

Many interesting factors can explain the variety of the situations described in the previous paragraph. The first of these factors is the variability of the assay used to measure serum or plasma creatinine (13, 21, 24). The assay used to measure creatinine is the alkaline picrate method or Jaffe method. This colorimetric assay measures the interaction of creatinine with picrate under alkaline conditions, which leads to a change in color of the solution that is proportional to the amount of creatinine in the sample. We can measure the UV absorption of light at 530 nm of wavelength and translate that reading to a concentration of creatinine with the help of a standard curve relating UV absorption to concentration of creatinine. The major disadvantage with this assay is that creatinine is not the only substance that can interact with picrate under alkaline conditions. Many other substances that we can find in serum or plasma (like proteins, glucose, cephalosporines, ketones, urate, acetone, and pyruvate) can reduce picrate under alkaline conditions and form colored substances. These noncreatinine chromogens result in an increase in serum creatinine measured by this method that can reach a 20% overestimation of the real value. This overestimation effect will be higher when the renal function is normal and it will be less when the renal function is decreased. This is so, because when the renal function is normal the noncreatinine chromogens will account for a higher relative amount when compared to the creatinine amount in the sample, which increases the chances of the noncreatinine chromogens of interacting with picrate. When the renal function is decreased then creatinine is accumulated but not the noncreatinine chromogens, which will decrease the probability that a noncreatinine chromogen will interact with picrate. Another drawback of the Jaffe method is negative interference from high concentrations of bilirubin or other compounds in the serum of jaundiced patients. They are called negative interference substances because they interact with picrate but don't cause a change in color of the solution. The mechanism of this interference is incompletely understood.

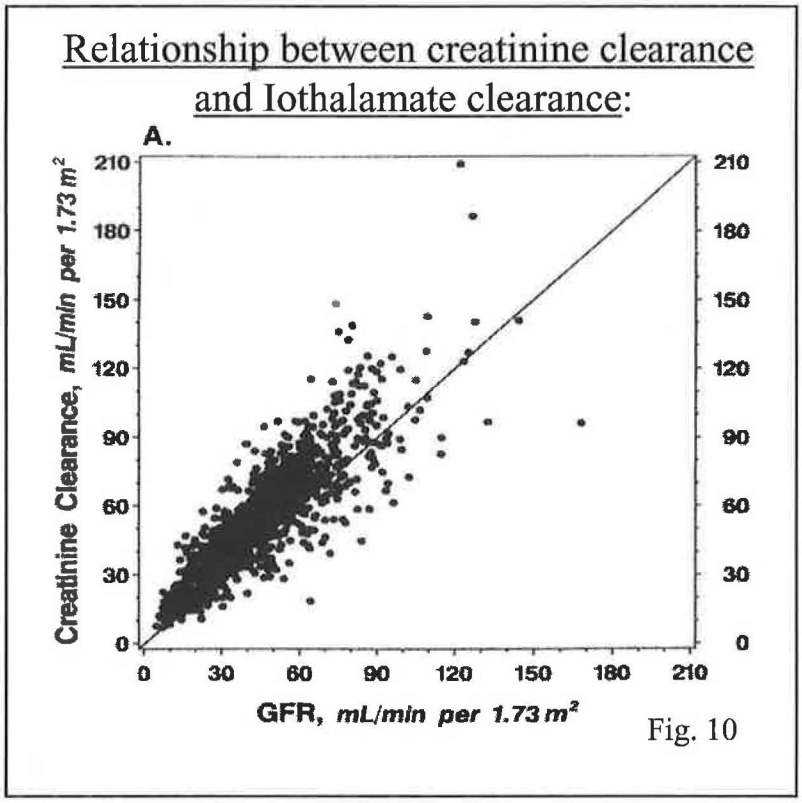


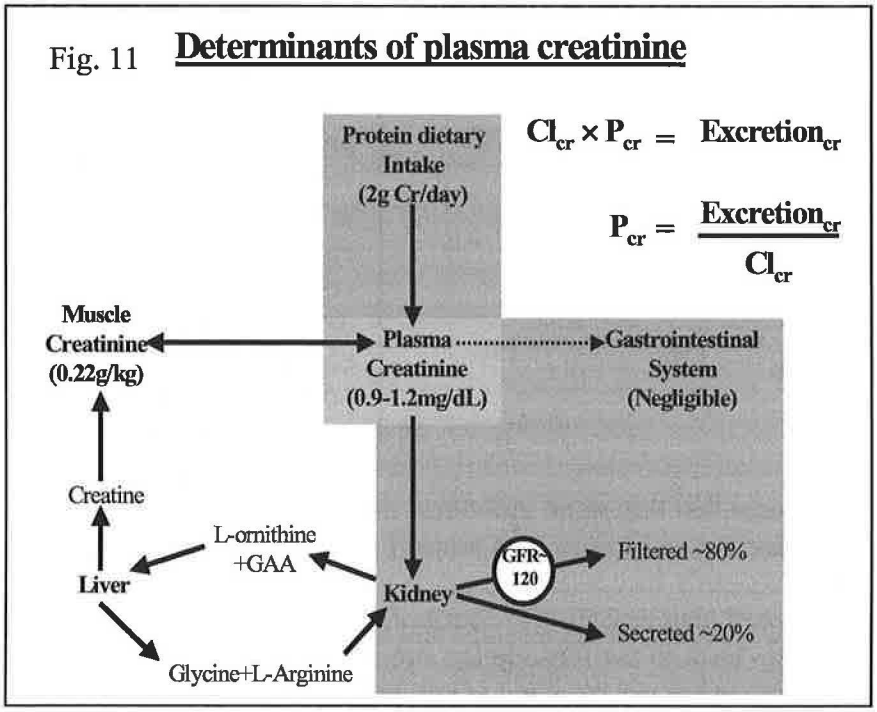
Fig. 10

The simple alkaline picrate method has been modified to resolve this interference with other noncreatinine chromogens. One of these modifications is the kinetic alkaline picrate method. The kinetic alkaline picrate method takes advantage of the differential rate of color development for noncreatinine chromogens vs that for creatinine. Creatinine changes the color of the solution very fast fashion and if this velocity can be differentiated by the autoanalyzer reading the UV absorbance, then we should be able to get rid of the non-specific interactions allowing a rate-dependent separation of creatinine from noncreatinine chromogens. However, many of the noncreatinine chromogens can have a rate of color development equal to that of creatinine, making it impossible for this modification of the alkaline picrate method of eliminating all possible positive interferences. Glucose and uric acid reduce picrate at a very slow velocity and the autoanalizer wont be able to read them as creatinine. Moreover, high concentrations of bilirubin still can reduce substantially the measured creatinine concentration, as this modification of the assay has no impact over the negative interference substances.

The enzymatic method is based on the reaction of creatinine with the enzyme iminohydroxylase (creatinine deaminase) to form ammonia and N-methylhydantoin. The ammonia formed is quantified by reaction with bromophenol blue and translated into creatinine concentration. This assay can more efficiently get rid off a larger portion of the positive interferences resulting from noncreatinine chromagens, however, serum glucose concentrations > 100 mg/dL and bilirubin concentrations of 7-18 mg/dL can still produce negative interference, thereby reducing the measured creatinine by ~ 15%.

So far we can say that the reported value for plasma creatinine may be an overestimate or a underestimate of the real value depending on the assay used to measure plasma creatinine, the presence or absence of other non creatinine chromogens with positive interference, the presence of substances with negative interference and what the real renal function level is at the moment of measurement.

Fig. 11 **Determinants of plasma creatinine**



In addition to what has been discussed before, the serum creatinine is the net result of multiple factors that mainly determine the balance of how much creatinine is added to the body and how much is excreted from the body (13, 14) (see Fig. 11). Addition of creatinine mainly happens by the intake of protein in the form of meat. When we eat cooked meat, we in fact ingest a

combination of creatine (the precursor of creatinine) and creatinine, as 50-60% of the creatine in muscle is converted to creatinine by a nonenzymatic hydrolysis reaction that happens to be enhanced when meat is heated. The creatine will be absorbed in the intestine and once in plasma it can be stored in the muscle cells or it can be excreted by the kidneys. Creatinine will also get absorbed from the gut and then it will be filtered and secreted by the kidney. Also, creatine is synthesized by the liver by a process that involves metabolic communication with the kidney, then it is stored in the muscle. In the muscle 2% of the stored creatine will get converted into creatinine by means of a nonenzymatic hydrolysis reaction. Once creatinine is formed, it can leave the muscle and get to the blood from where it is filtered and secreted in the kidney. As you can see, the major way of excretion of creatinine is glomerular filtration and renal tubular secretion. The gut excretion is negligible under normal circumstances but it can become a significant creatinine excretory route when the renal function is significantly decreased.

The determinants of plasma creatinine can be simplified to those processes that determine production (namely liver creatine synthesis, muscle generation of creatinine from creatine, and dietary meat intake), and those that determine excretion (under most of the circumstances renal function). We could state it using the mathematical formula for the concept of clearance. In other words, the plasma concentration of creatinine equals excretion of creatinine divided by clearance of creatinine. If the individual is in steady state, then excretion equals production so either production of excretion of creatinine can be used in the numerator. Using this concept, lets review a couple of examples of how these factors can determine what the plasma creatinine level will be.

Determinants of plasma creatinine

Body Builder

Fig. 12

Cl_{cr}	$[P_{cr}]$	$Excretion_{cr}$	$Production_{cr}$
100	1.0	100	100
100	1.5	150	150

Lets start with the normal individual in steady state (see Fig. 12). This normal individual produces 100 of creatinine. Because he is in steady state, production equals excretion of creatinine. Lets determine that his clearance of creatinine is 100. Going back to the formula, we can calculate that the concentration of creatinine will be 1. Lets say now that this same individual now increases production to 150 because he is a body builder and he likes to load himself with creatine supplements and high meat intake and subsequently increases his muscle mass by 50%. When he reaches steady state, excretion will also be 150. He continues to have a creatinine clearance of 100. Now, if we go back to the formula the concentration of creatinine goes up to 1.5. Notice that the clearance has not changed. Only production went up. The

increase in concentration of creatinine can erroneously lead us to think that the renal function has decreased when it has not (see Fig 12).

The second example is aging. In the top of the figure 13 we have a young healthy lady in steady state in whom production and excretion of creatinine equals 100. Her clearance is 100 and after our calculation we can get her creatinine concentration is 1.0. The same lady ages, losses muscle mass and changes her dietary habits, so that her creatinine production decreases to 60. After some time she will reach steady state and excretion of creatinine will be 60. As a result of decreases of clearance of creatinine related to the aging process, her clearance is now 75. After our calculation is done the concentration of creatinine will be 0.8. A concentration of creatinine that we generally do not associate with decreased renal function, but in fact there has been a decrease in renal clearance. We don't see a noticeable increase in the concentration of creatinine basically because both the production of creatinine and the clearance of creatinine decreased proportionally in relation to the aging process.

Determinants of plasma creatinine

Fig. 13 *Elderly*

	Cl_{cr}	$[P_{cr}]$	$Excretion_{cr}$	$Production_{cr}$
	100	1.0	100	100
	75	0.8	60	60

My third example is the malnourished individual (shown in Fig. 14). We start with the same healthy female in steady state. She develops anorexia. She drastically decreases the intake of protein in the form of meat, she losses muscle mass so her production of creatinine goes down to 50. When she achieves steady state, the excretion of creatinine will also go down to 50. Her clearance of creatinine stays the same. After our calculation, her

Determinants of plasma creatinine

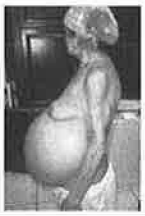
Fig. 14 *Malnourished*

	Cl_{cr}	$[P_{cr}]$	$Excretion_{cr}$	$Production_{cr}$
	100	1.0	100	100
	100	0.5	50	50

Determinants of plasma creatinine

Chronic liver disease

Fig. 15

	Cl_{cr}	$[P_{cr}]$	$Excretion_{cr}$	$Production_{cr}$
	100	1.0	100	100
	100	0.6	60	60

concentration of creatinine will be 0.5. In this hypothetical case the clearance of creatinine has to go down to 50 or less before we may start noticing an increase in the concentration of creatinine that we will associate more with the possibility of having decreased clearance.

My fourth example is the patient with chronic liver disease (shown in Fig. 15). Here we also start with the healthy patient in steady state with the same baseline characteristics as in the previous examples. She now develops severe chronic liver disease. Her meat dietary intake decreases, she losses muscle mass, and her liver wont be able to synthesize creatine efficiently, so her production of creatinine will decrease from 100 to 60. After achieving steady state the excretion of creatinine will equal production of 60. With the help of the formula we can determine her concentration of creatinine will be 0.6. The clearance of creatinine has to come down to 50 or less before a noticeable increase in the concentration of creatinine is observed (20).

Determinants of plasma creatinine

Chronic Renal Failure

Fig. 16

	Cl_{cr}	$[P_{cr}]$	$Excretion_{cr}$	$Production_{cr}$
	100	1.0	100	100
	40	2.5	100	100

My last example is the individual that develops renal failure (see Fig. 16). We start with the healthy, normal renal function individual in steady state with the same baseline characteristics as in the previous examples. The individual develops renal failure but the production of creatinine stays the same. Once the individual achieves steady state, the excretion of creatinine will be 100. With the help of our formula, we can see that the concentration of creatinine will be 4. The concentration of

creatinine went up because the production of creatinine was unchanged, but the clearance of creatinine decreased as a result of renal failure.

These examples also emphasize again the fact that the concentration of the marker of GFR is not a good way of estimating renal function.

Fig. 17 shows how inaccurately the concentration of creatinine can estimate GFR. An increase in the serum creatinine level from 1 mg/dL to 2.0 mg/dL is consistent with a 50% decrease in GFR, but an increase of serum creatinine from 5 mg/dL to 6 mg/dL is associated with a very small decrease in GFR. The same is true for plots of the inverse of the serum creatinine against GFR measured by iothalamate clearance. The inverse of the serum creatinine is mostly an overestimation of GFR and the accuracy of the prediction is very poor specially in the setting of preserved renal function.

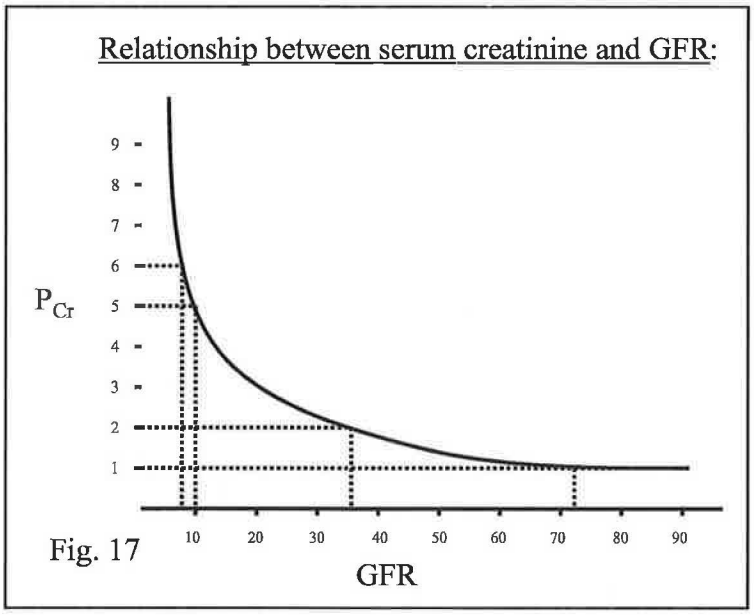


Fig. 17

So far, we have discussed many of the factors that can contribute to the inaccuracy of the measured creatinine clearance as an estimator of glomerular filtration rate. Is there any modification to the procedure we can use to improve the accuracy of the estimate of GFR? The answer is yes. The first modification to the procedure is measuring a creatinine clearance after the patient has been

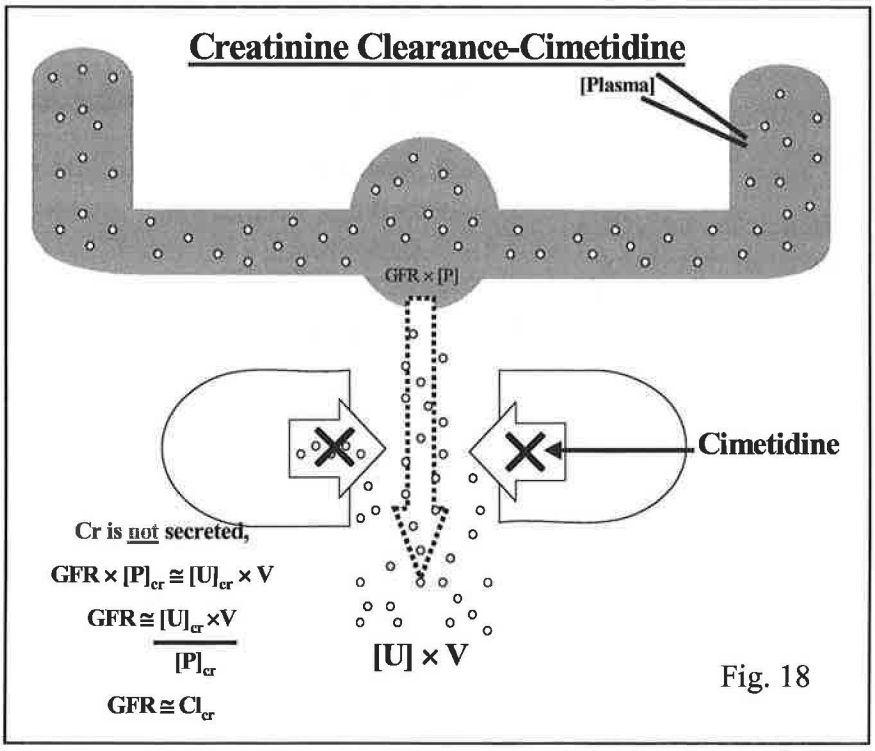


Fig. 18

treated with cimetidine (see Fig. 18). Cimetidine inhibits the secretion of creatinine by the renal tubules and consequently will make creatinine behave as a GFR marker that does not gets reabsorbed or secreted and will approximated GFR more accurately (15, 16). The problems with this approach are several. In the first place the data available in the medical literature consist of very small groups of patients. Second every single one of these small studies used a different dose and regimen for the administration of the cimetidine before the creatinine clearance was actually done, so the studies are not easy to compare. These different regimens of administration of cimetidine lead to different levels of inhibition of the renal tubular creatinine secretory component.

<u>Relationship between urea clearance and Iothalamate clearance</u>:

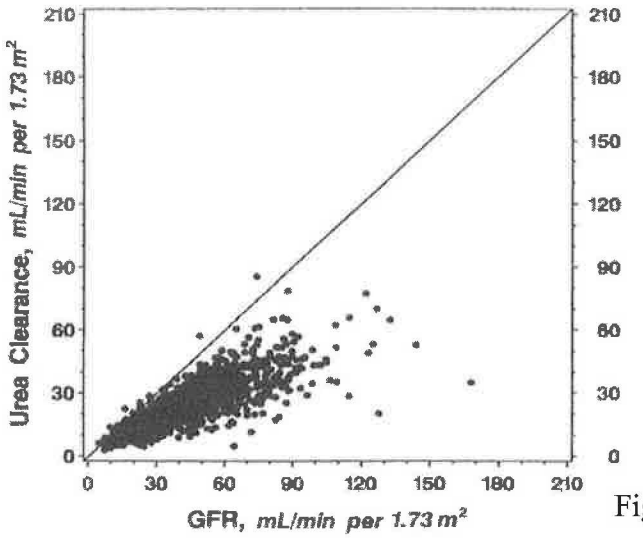


Fig. 19

Another modification to the procedure of creatinine clearance that can improve the estimation of GFR derives from the use of a measured urea clearance (18, 46). Urea gets reabsorbed in the renal tubules as shown in Fig. 6. As a result of this, urea clearance is an underestimation of GFR. This is also shown in Fig. 19. By itself this is not going to be very helpful. But if we measure creatinine clearance and urea clearance, add the respective values and get the mean of them as shown in Fig. 20, we will be very probably estimating GFR more accurately. Fig. 10 shows how a measured creatinine clearance compares to GFR measured by iothalamate clearance.

<u>Mean of creatinine and urea clearance</u>:

If creatinine is secreted,	**If urea is reabsorbed,**
$GFR \times [P]_{creat} < [U]_{creat} \times V$	$GFR \times [P]_{BUN} > [U]_{urea} \times V$
$GFR < \frac{[U]_{creat} \times V}{[P]_{creat}}$	$GFR > \frac{[U]_{urea} \times V}{[P]_{BUN}}$
GFR < Creatinine Clearance	**GFR > Urea Clearance**
"Overestimation of GFR"	**"Underestimation of GFR"**

$$\frac{\text{Creatinine clearance} + \text{Urea clearance}}{2}$$

Fig. 20

$$Cl_{urea} < GFR < Cl_{Cr}$$

Most of the data points are above the identity line clearly showing the overestimating trend of measured creatinine clearance. Fig. 19 shows the relationship of measured urea clearance and GFR measured by iothalamate clearance. Most of the data points in this case are below the identity line clearly showing the underestimating trend of the measured urea clearance. However, when we compare the mean of measured creatinine and urea clearance with GFR measured by iothalamate clearance we can see how the data points move closer to the identity line clearly evidencing the improvement in the estimation of GFR (see Fig. 21).

Relationship between mean of creatinine and urea clearance and Iothalamate clearance:

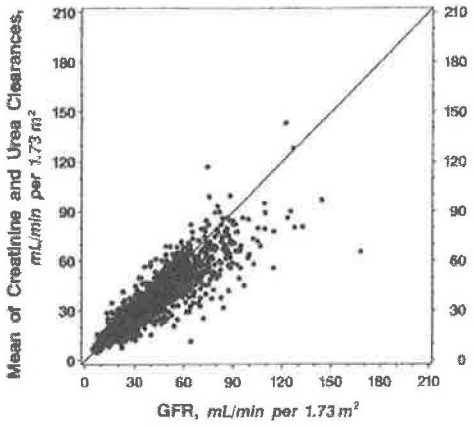
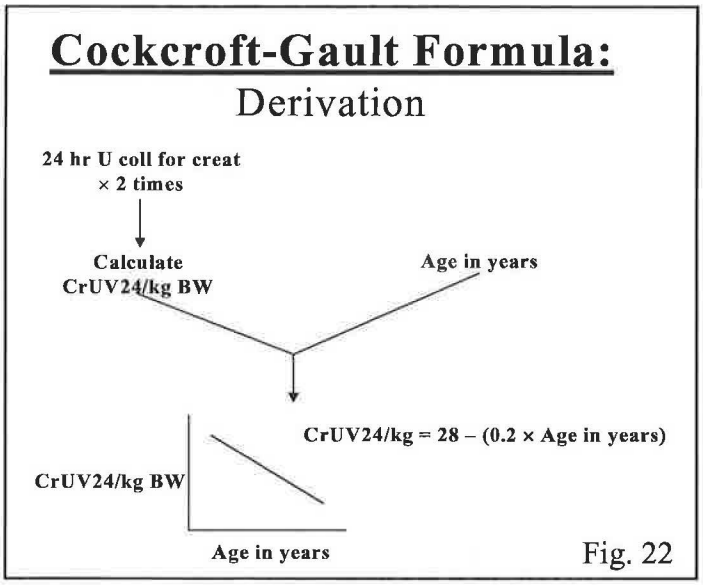


Fig. 21

So we found 2 possible ways of improving our estimate of GFR. There is still a problem that consists in determining the accuracy of the urine collection in the 24 hrs period. The common way of determining the appropriateness of the 24 hr period collection is comparing the actual amount of creatinine collected in the volume of urine at the end of the collection period, with the expected urinary creatinine excretion in a period of 24 hrs calculated by kg of actual body weight. This is traditionally calculated using a 24 hours urine creatinine excretion rate of 15-20 mg/kg in women and 20-25 mg/kg in men. But these are the estimated urinary creatinine excretory rates for healthy, steady state and fit men and women. We can not extrapolate the use of these values to the variety of clinical situations we encounter in our practice every day. As shown in Table 3 the urinary excretion of creatinine is age and sex dependent. The urinary excretion of creatinine is lower in women compared to men, and it decreases with age in both men and women. Other demographic parameters can affect the urinary excretion of creatinine like muscle mass, dietary habits, medical conditions (severe malnutrition, chronic liver disease, obesity, pregnancy, diabetes mellitus, etc). For this reason we need another way of approximating GFR that does not require a 24 hr

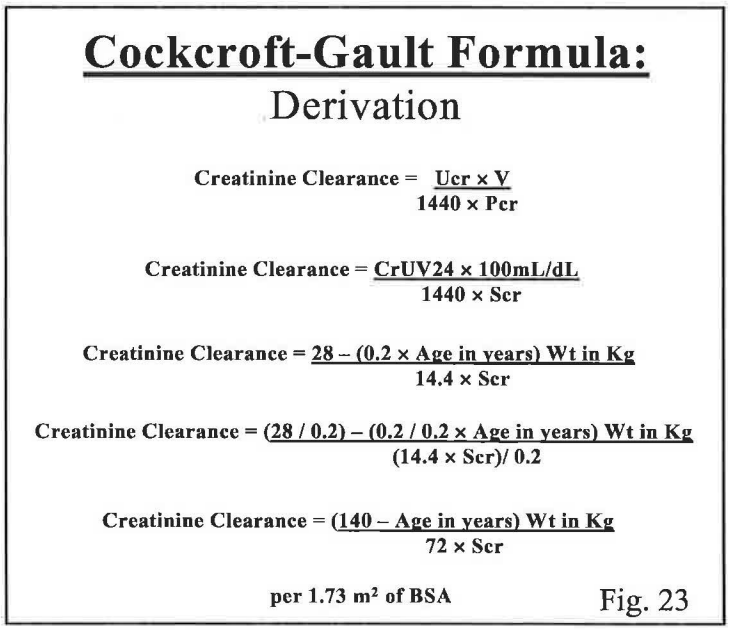
TABLE 3. *Plasma creatinine and creatinine excretion in adults at different ages*

	Age (years)	Serum creatinine (mg/dl ± SD)	Urinary creatinine (mg/kg/24 hr ± SD)	C_{crea} (ml/1.73 m²)
Males	20–29	0.99 ± 0.16	23.8 ± 2.3	110
	30–39	1.14 ± 0.22	21.9 ± 1.5	97
	40–49	1.10 ± 0.20	19.7 ± 3.2	88
	50–59	1.16 ± 0.17	19.3 ± 2.9	81
	60–69	1.15 ± 0.14	16.9 ± 2.9	72
	70–79	1.03 ± 0.22	14.2 ± 3.0	64
	80–89	1.06 ± 0.25	11.7 ± 4.0	47
	90–99	1.20 ± 0.16	9.4 ± 3.2	34
Females	20–29	0.89 ± 0.17	19.7 ± 3.9	95
	30–39	0.91 ± 0.17	20.4 ± 3.9	103
	40–49	1.00 ± 0.24	17.6 ± 3.9	81
	50–59	0.99 ± 0.26	14.9 ± 3.6	74
	60–69	0.97 ± 0.17	12.9 ± 2.6	63
	70–79	1.02 ± 0.23	11.8 ± 2.2	54
	80–89	1.05 ± 0.22	10.7 ± 2.5	46
	90–99	0.91 ± 0.12	8.4 ± 1.4	39



urine collection.

This was one of the reasons why Cockcroft and Gault in Canada performed the studies that led to the derivation of their formula to estimate creatinine clearance (11). They studied 249 hospitalized male patients with normal serum creatinine in the age range of 18-92 years old. They got two 24hrs urine collections on each one of the patients and measured serum creatinine using the kinetic alkaline picrate method. They calculated the 24 hr urinary creatinine excretion per kg of actual body weight and plotted this against age. After getting the regression line that would related the two parameters, they determined the mathematical equation that would define the regression line. They then substituted this mathematical equation into the numerator of the formula used to calculate a creatinine clearance from the data coming from a 24 hr urine collection. They resolved the equation to the simplest terms and got the formula that we are very familiar with (see Fig. 22 and 23). Then they plotted the estimated creatinine clearance using the formula against the measured creatinine clearances of the same individuals used to get the formula Fig. 24. For the purpose of comparison Fig. 25 shows the relationship of the estimated creatinine clearance by Cockcroft-Gault formula with GFR measured by iothalamate clearance. One of the drawbacks of this formula is that it estimates creatinine clearance and not GFR. Another drawback is that this formula was validated in the same population where it was derived. Also we don't know much information about the patients in the study. There was no female representation in the study and to account for the in the formula the investigators decided to multiply the estimated creatinine clearance value by 0.85 as females muscle mass is approximately 15% lower than in males (23, 27).



If we carefully analyze the Cockcroft-Gault formula we can notice that it is just another mathematical formula to express the concept of creatinine clearance (see Fig. 26). If you recall our previous discussion, creatinine clearance equals, excretion of creatinine divided by plasma concentration of creatinine. Excretion of creatinine, in the numerator of the formula, is the same as urinary excretion of creatinine, that is, $U_{creat} \times V$. In the formula, excretion of creatinine is equal to (140-Age) Body weight in kilograms. The denominator in both formulas is equal to the plasma concentration of creatinine multiplied by a constant that helps express the final product in units of ml/min. The excretion of creatinine in the clearance formula is determined by the accuracy of the 24 hr urine collection. The excretion of creatinine in the Cockcroft-Gault formula is determined by the accuracy of the estimation of urinary excretion of creatinine by demographic data like age and actual weight. A measured creatinine clearance requires a urine and a plasma sample for its calculations. The Cockcroft-Gault formula just needs one plasma sample for its calculations. Both formulas are subjected to the inaccuracies of the assays available to measure serum creatinine and both are affected by the multiple factors that determine the serum creatinine level, basically the balance in between production and clearance of serum creatinine. Up to this point the need for a more accurate formula to estimate GFR instead of creatinine clearance was evident.

Relationship between predicted creatinine clearance and measured creatinine clearance:

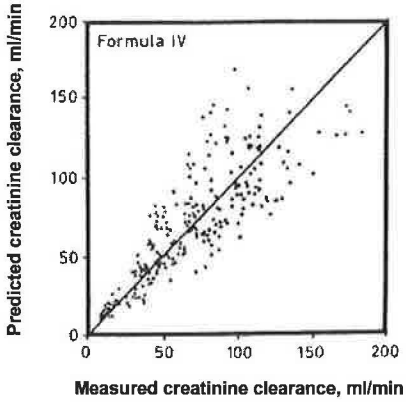


Fig. 24

Relationship between predicted creatinine clearance and Iothalamate clearance:

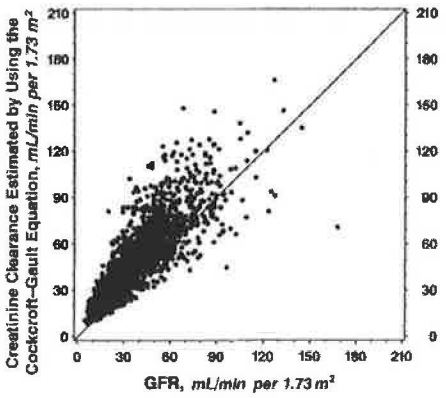


Fig. 25

Cockcroft-Gault Formula:

How does it compare to measured Cl_{cr} ?

Fig. 26

$$Cl_{cr} = \frac{\text{Excretion of Cr}}{P_{cr}} \qquad Cl_{cr} = \frac{(140\text{-Age})\ Wt}{72 \times P_{cr}}$$

MDRD Formula:

Derivation

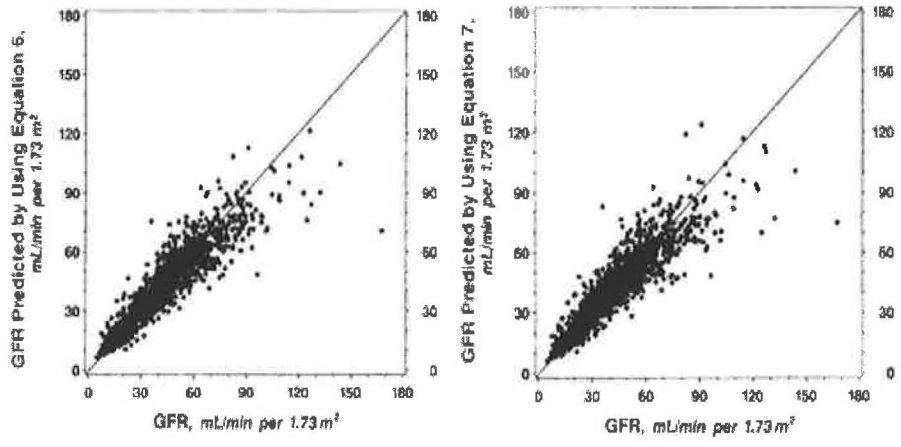
1628 pts
MDRD study

Age 50.6 ± 12.7 years
Weight 79.6 ± 16.8 kg
BSA 1.91 ± 0.23 m2
GFR 39.8 ± cc/min/1.73m2
Cr Cl 48.6 ± cc/min/1.73m2
Serum creatinine 2.3 ± mg/dL
Male 60%
White 88%
Diabetics 6%
GN 32%
TIN 7%
Unknown 40%

Fig. 27

It was not until 1999 when the MDRD study group was able to develop a new prediction formula for GFR (18). These investigators obtained a sample of 1628 patients from the MDRD study. The patient characteristics are described in figure 27. In general they were mostly white, non diabetic, underrepresented with minority groups, mostly unknown etiology for renal failure. They divided the original sample of patients in two groups. One group they called the training sample which they used to derive the formula and the validation group which the investigators used to validate the formula. The investigators used a multiple variable logistic regression model in the training sample to determine a set of variables that jointly predicted GFR. A P value less than 0.001 was used as the criterion for entry of a variable into the model. From all the variables considered, only serum creatinine, age, race, sex, plasma BUN and serum albumin entered finally the model. Consequently the predictive equation is a multiplicative of variables that predicted GFR with statistical significance. Once the formula was generated, it was used to estimate GFR in the validation group. Then the estimated GFR was plotted against the measured GFR by iothalamate clearance and Fig. 28. Later on, using a similar approach an abbreviated version of the formula was introduced to clinical practice. This abbreviated formula includes the following variables: serum creatinine, age, sex and race. Since its introduction to clinical practice the MDRD formula has been validated in hypertensive African Americans and other populations however, the performance is lower than with populations different from which it was derived and originally validated.

Fig. 28 Relationship between predicted GFR and Iothalamate clearance:



The Cockcroft-Gault and MDRD formulas that we have described are better estimates of creatinine clearance and GFR, respectively, than serum creatinine or inverse of serum creatinine alone. However, they still have a considerable degree of inaccuracy (23, 27). For this reason we have continued to look for better estimators and/or better markers of GFR. One of these new markers is cystatin C. As shown in Fig. 29, Cystatin C is a 13 kDa protein of 122 amino acids produced by a housekeeping gene to function as a cysteinase protease inhibitor. It is produced in all the nucleated cells of the body at a constant rate. It is secreted into the blood, from which it is filtered in the glomerulus, then it is reabsorbed by the proximal tubule cell, where it is degraded. Only less than 1% of it escapes the reabsorption and degradation steps. Glucocorticoids, HIV infection, pregnancy state, and hyperthyroidism are the only known situations where production of cystatin C is enhanced and maybe use of cystatin C as a marker of GFR might have to be analyzed very

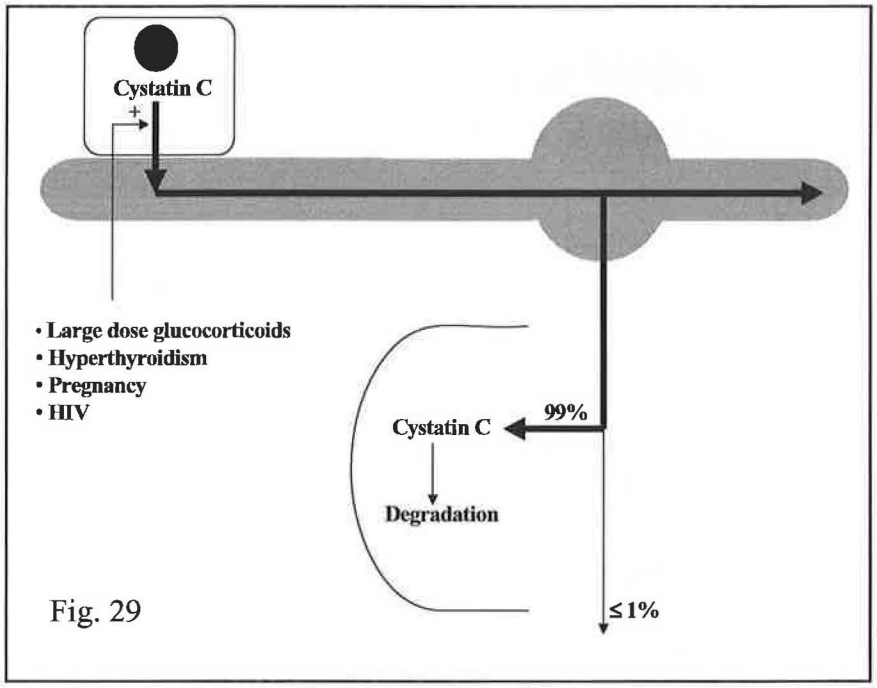
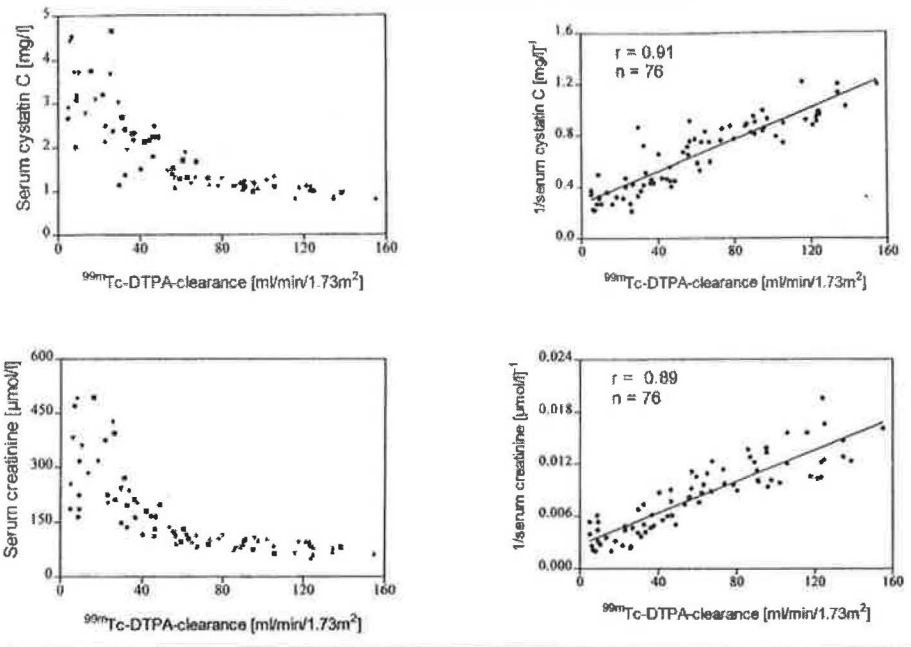


Fig. 29

Fig. 30 Relationship of plasma creatinine and cystatin c To 99m Tc-DTPA clearance:



carefully (30, 31, 33, 35, 36, 37, 43, 45).

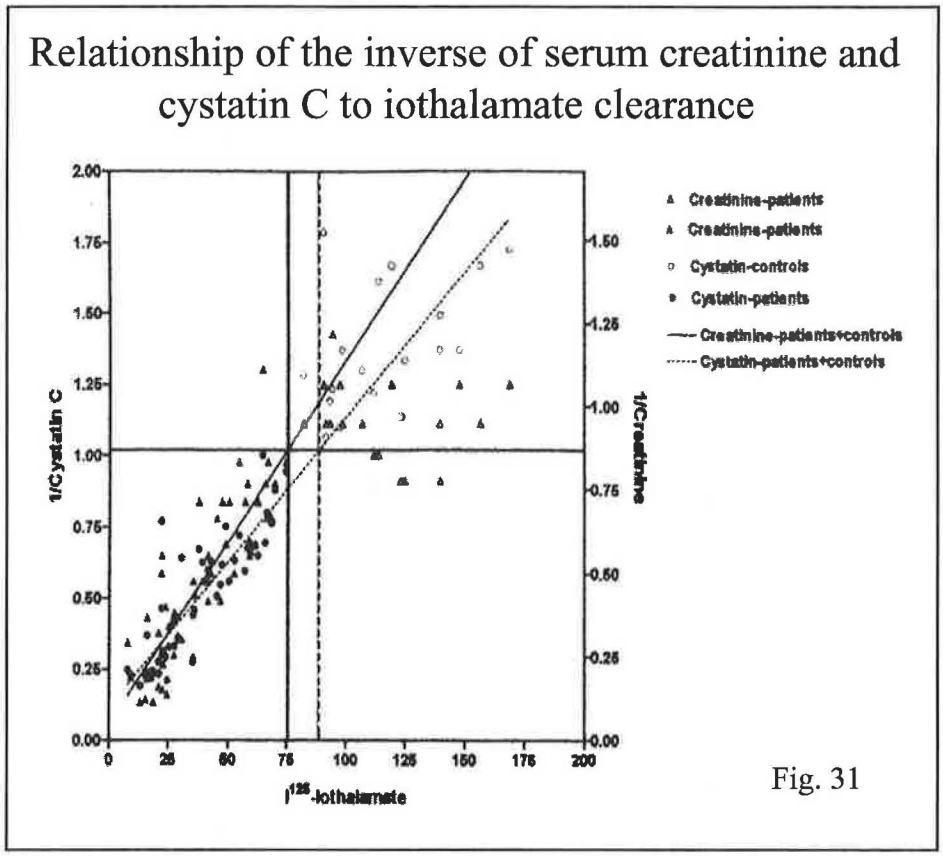


Fig. 31

As you see in Fig. 30, when either serum cystatin C or inverse of serum cystatin C is plotted against GFR, they look almost exactly to the analogous plots using serum creatinine or inverse of serum creatinine. The correlation coefficients of levels of the inverse of serum cystatin C with GFR is higher than the correlation of inverse of serum creatinine with GFR. One of the advantages of levels of cystatin C over serum creatinine is that cystatin C appears to be an earlier marker of GFR than serum creatinine as shown in Fig. 31. One of the possible disadvantages of cystatin C has to do with the variability of the assay used to measure serum levels of cystatin C.

Fig. 32A shows the mean and range of values for serum creatinine measured using the kinetic alkaline picrate assay in twelve normal renal function individuals. As you can see the assay has a lot of interindividual variation but little intraindividual variation (32, 41). This is a good characteristic of the kinetic alkaline picrate assay as this allow us to be able to follow the serum creatinine along with time as a marker of renal dysfunction, as the values in the same individual are not going to be significantly different from each other.

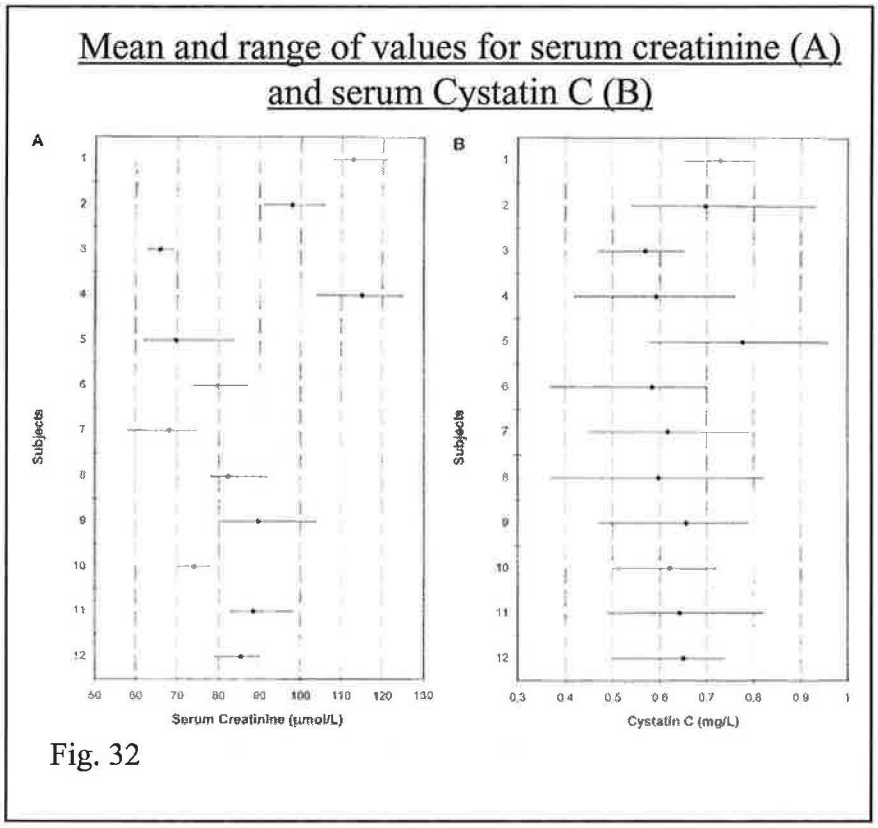


Fig. 32

However this can also explain why individuals with low normal range serum creatinine values may have renal dysfunction even when their serum creatinine levels increase but stay in the normal range value (34, 42, 44). Fig. 32B shows the mean and range of values for serum cystatin C levels measured by a turbidimetric assay in twelve normal individuals. You can notice that the assay has little interindividual variation but a lot of intraindividual variation. This could explain why the serum level of cystatin C can go up more obviously with smaller changes in renal function and this could be a clear advantage over serum creatinine.

So far, we can say that inulin meets most of the characteristics of an ideal GFR marker. Although it has remained the gold standard, it is not practical for routine use due to its limited supply and difficulty of measurement. Although clearance techniques are more accurate than serum creatinine or inverse of serum creatinine, the appropriateness of the 24 hr urine collection still is a common problem. The estimation formulas are derived and validated in the same populations where they were created and will need to be validated in other innumerable populations as their performance in populations different than the ones where they were derived is lower. Cystatin C so far can be seen as a refined serum creatinine that can pick up decreases in GFR earlier than serum creatinine (38, 39, 40). In the meantime, while we await to get more accurate estimators of GFR, we may be misclassifying patients into the different CKD stages and, more important than that, calculating a GFR that is probably far from the real value consequently exposing patients to nephrotoxic doses of drugs that can result in new renal damage or additional damage and its associated morbidity. For this reason we are in need of more individualized and accurate ways of estimating GFR.

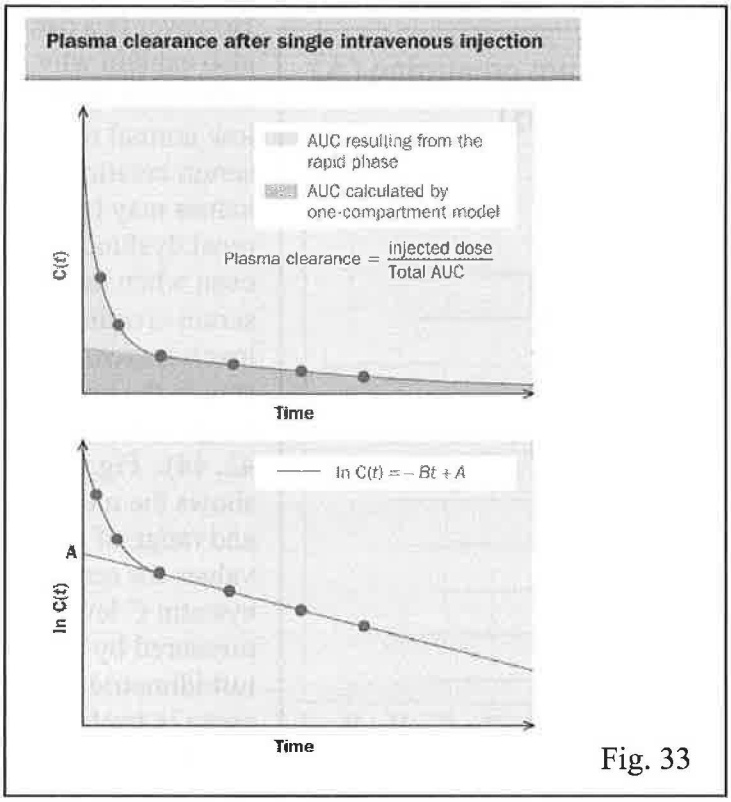


Fig. 33

One of these more individualized ways of getting an estimate of GFR more accurately is the single injection technique. With this technique a bolus injection of one of the exogenous markers of GFR is given to the patient. Then, the disappearance curve of the plasma concentration of the marker is constructed. This curve has two components: a rapid phase reflecting the distribution of the marker from the intravascular space to the extravascular space, and a slow phase reflecting the renal excretion of the marker. A two-compartment model is required to describe the entire plasma disappearance curve for the marker, but it requires frequent blood sampling, which is impractical in most of the clinical scenarios. Therefore, a one-compartment model is widely used which uses only the slow phase of the disappearance curve for the marker (47, 48). The mathematical expression of this model is shown in Fig. 33 where C(t) is the plasma concentration of the marker at a given time, A is the zero-time intercept, and –B is the rate constant for the decrease of the plasma concentration of the marker in a semilogarithmic plot, that is the result of the renal excretion of the marker.

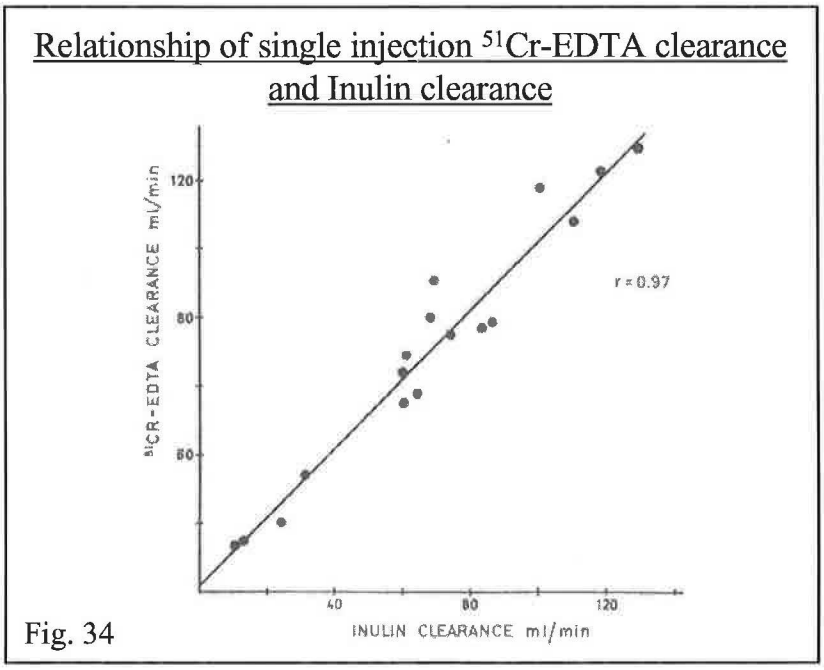


Fig. 34

The line can usually be determined by two blood samples during the slow phase (90 and 120 min after injection) although a relatively long time (3-24hrs) may be required to obtain the accurate falling slope of the marker in patients with impaired renal function. Plasma clearance is

slightly overestimated with this technique, since it does not account for that part of the AUC resulting from the rapid phase.

Fig. 34 shows the relationship of single injection ^{51}Cr-EDTA clearance to Inulin clearance in studies performed by Bröchner-Mortensen and colleagues. Although a very small group of patients, it seems that the single injection ^{51}Cr-EDTA clearance has a higher correlation with GFR estimated by inulin clearance however the procedure still requires two timed blood samples.

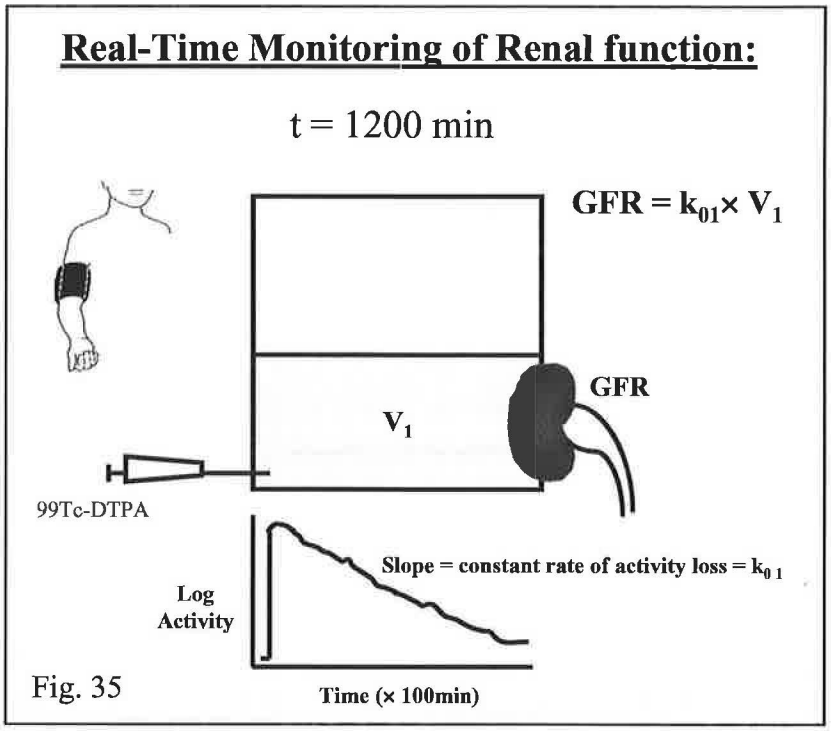


Fig. 35

This is why Rabito and colleagues did in the early 90's in Harvard (49). They had designed a nuclear medicine based technique to estimate GFR in an accurate and individual manner (see Fig. 35). The procedure consist in the intravenous injection of ^{99}Tc-DTPA bolus followed by a 45 min of equilibration of the distribution of this radiotracer into the intravascular and interstitial volume. Then a radioactivity detector that they called the ambulatory renal monitor (ARM) is wrapped around the upper arm of the patient and detection of radioactivity is started. The rate of loss of radioactivity is plotted in relationship with time. Then the slope of the tracing, or rate constant of loss of radioactivity is calculated. The rate constant of loss of radioactivity is going to be

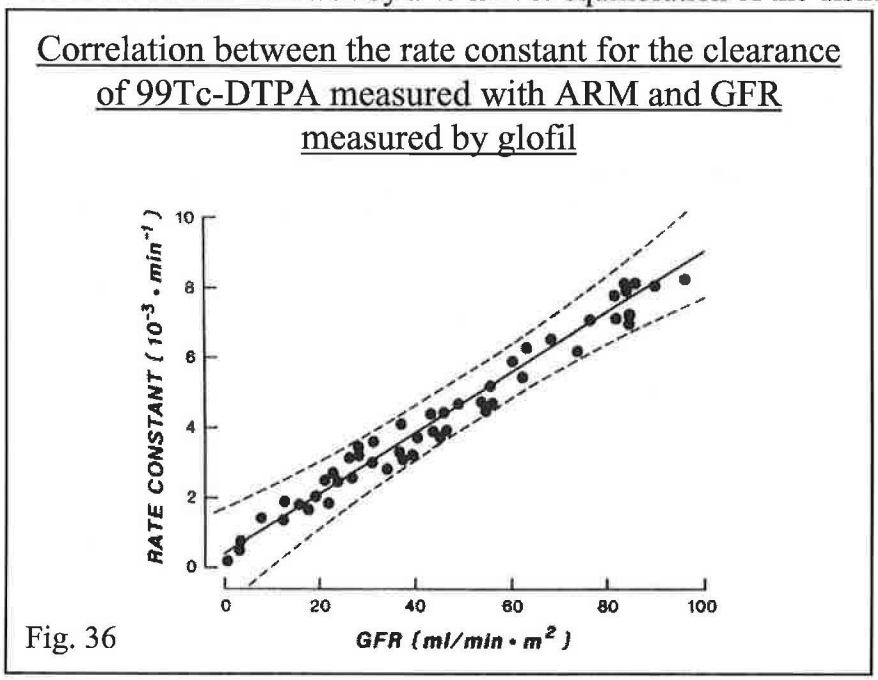


Fig. 36

determined by the GFR. That is, the higher the GFR, the higher the rate constant of loss of radioactivity, and vice versa. The rate constant of loss of radioactivity correlates very closely with GFR measured by iothalamate clearance using the constant infusion technique as shown in Fig. 36. This is a procedure that can be done in an ambulatory basis, in hospital and in the ICU setting and would be an individual and accurate estimation of GFR for any patient and any clinical situation.

To better show you the value of this technique Fig. 37 shows you here different clinical situations in which the ARM was used to assess renal function. The figure to the upper left shows you an individual with stable renal function evidenced by an unchanged and stable rate constant decrease in activity after an angiogram . The figure 36 shows you a patient that had a coronary angiograp hy that resulted in decrease in renal function and mild elevation in the serum creatinine. Fig is showing a patient admitted to ICU who dropped the BP after which the renal function decreased significantly. These and many other examples of the use of ARM are available in the literature not only for the intensive care unit setting but in the ambulatory outpatient setting. Interestingly the changes in the GFR noticed with this technique is as close as we can get to real time GFR estimation and in many situations the change in GFR noticed by this technique preceded the increase in the plasma creatinine and changes in urinary output, which are the most common currently used signs of renal dysfunction or insult.

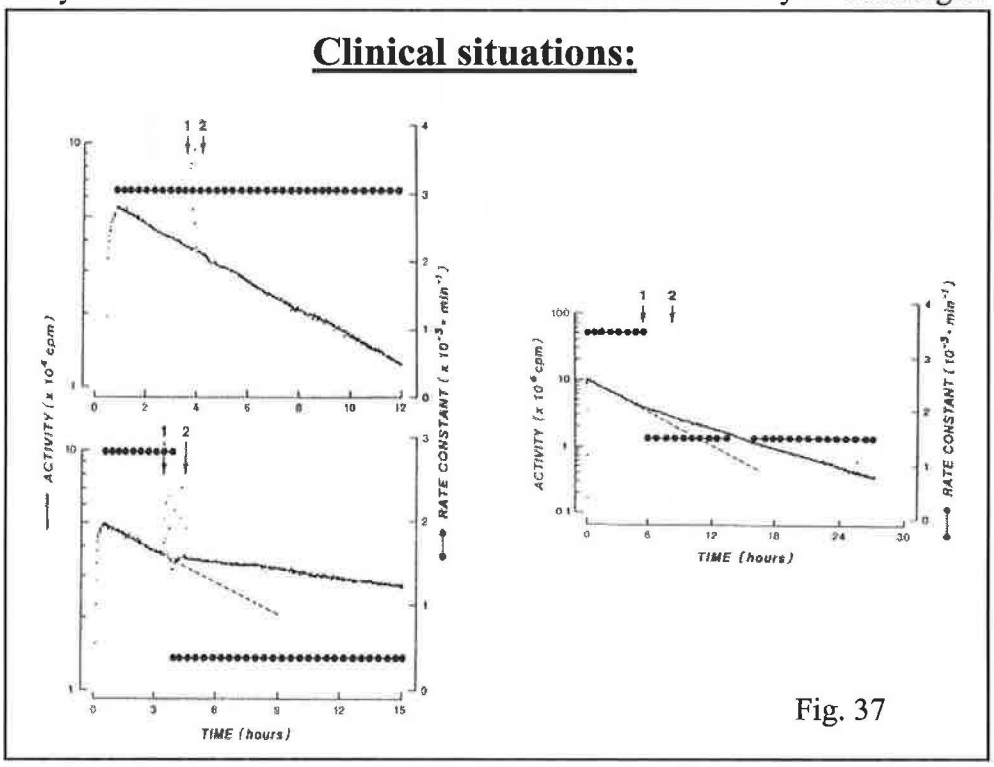


Fig. 37

To conclude, plasma creatinine and inverse of plasma creatinine are very poor markers or estimators of glomerular filtration rate. The commonly used creatinine clearance is an overestimator of GFR most of the time and its accuracy is determined by the accuracy of the collection. Creatinine clearance can approximate GFR more accurately if the collection is done after dosing the patients with cimetidine and/or by calculating a urea clearance and getting a mean of creatinine and urea clearance; however, both require an accurate urine collection, and the absence of a complete urine collection subtracts significant accuracy to these methods. The Cockcroft-Gault formula

is an estimate of creatinine clearance, not GFR, and it should be corrected for body surface area. It is very inaccurate at normal renal function levels and is more accurate as renal function decreases. In spite of being inaccurate, it is a better estimate of GFR then plasma creatinine and is currently one of the recommended estimation methods by the DOQI guidelines. The MDRD formula estimates GFR, but its performance decreases when used in populations different to the one used to derive the formula, and it will be biased toward a lower GFR estimate when used to estimate renal function in patients with normal renal function. However, it is still recommended by DOQI for estimation of renal function. Cystatin C can be considered an enhanced and an earlier marker of GFR when compared to serum creatinine. The estimation of GFR can be individualized and its accuracy can be increased significantly with the use of methods like the single injection of exogenous markers of GFR or ambulatory renal monitoring by means of a single injection of 99Tc-DTPA and determination of the rate constant of activity loss when measured at the upper arm with the ARM. These techniques are considerably more expensive but offer the advantage of estimating a more accurate glomerular filtration rate than any of the previously discussed techniques. The ambulatory renal monitor can go a step further and, in addition to being very accurate, it can also provide a very close to real-time estimate of GFR in a variety of scenarios that include the outpatient, in hospital and intensive care settings.

References:

1. Brenner B, Rector F. Chapter 8 Glomerular ultrafiltration; 319-374. The Kidney, sixth edition.
2. Brenner B, Rector F. Chapter 44 Adaptation to nephron loss; 1901-1942. The Kidney, sixth edition.
3. Brenner B, Rector F. Chapter 52 Effect of aging on renal function; 2187-2218. The Kidney, sixth edition.
4. Johnson RJ, Fehally J. Chapter 3 Assesment of renal function; 27-34. Comprehensive clinical nephrology, second edition.
5. Johnson RJ, Fehally J. Chapter 7 Disorders of extracellular volume; 71-85. Comprehensive clinical nephrology, second edition.
6. Johnson RJ, Fehally J. Chapter 8 Disorders of water metabolism; 87-108. Comprehensive clinical nephrology, second edition.
7. McGonigle RJS, Wallin JD, Shaduck RK, Fisher JW. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. Kidney International 25: 437-444, 1984.
8. Seldin DW. The development of the clearance concept. J Nephrol 2004; 17: 166-171.
9. Richardson D, Speck D. Addressing students' misconceptions of renal clearance. Adv. Physiol. Educ. 28: 210-212, 2004.
10. Levey AS. Measurement of kidney function. Med Clin N Am 89: 457-473, 2005.
11. Gault MH, Longerich LL, Harnett JD, Wesolowski C. Predicting glomerular function from adjusted serum creatinine. Nephron 1992; 62:249-256.

12. Middleton J. UT Southwestern Glomerular filtration rate laboratory: Policies and procedures manual (Revised 9/24/1998)
13. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: New insights into old concepts. Clin Chem 38(10): 1933-1953, 1992.
14. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiological reviews 80(3): 1107-1213, 2000.
15. Burgess E, Blair A, Krichman K, Cutler E. Inhibition of renal creatinine secretion by cimetidine in humans. Renal Physiol 5: 27-30, 1982.
16. Kemperman FAW, Silberbusch J, Slaats EH, van Zanten AP, Weber JA, Krediet RT, Arisz L. Glomerular rate estimation from plasma creatinine after inhibition of tubular secretion: relevance of the creatinine assay. Nephrol Dial Transplant 14: 1247-1251, 1999.
17. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 16: 31-41, 1976.
18. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Annals of Internal Medicine 130(6): 461-470, 1999.
19. Verhave JC, Fesler P, Ribstein J, du Cailar G, Mimran A. Estimation of renal function in subjects with normal serum creatinine levels: Influence of age and body mass index. American journal of kidney diseases 46(2): 233-241, 2005.
20. Sherman DS, Fish DN, Teitelbaum I. Assessing renal function in cirrhotic patients: Problems and pitfalls. American journal of kidney diseases 41(2): 269-278, 2003.
21. Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, Levey AS. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. American journal of kidney diseases 39(5): 920-929, 2002.
22. Poggio ED, Nef PC, Wang X, Greene T, Van Lente F, Dennis VW, Hall PM. Performance of the Cockcroft-Gault and Modification of Diet in Renal Disease Equations in estimating GFR in ill hospitalized patients. American journal of kidney diseases 46(2): 242-252, 2005.
23. Agarwal R. Estimating GFR from serum creatinine concentration: Pitfalls of GFR-estimating equations. American journal of kidney diseases 45(3): 610-613, 2005.
24. Zuo L, Ma Y, Zhou Y, Wang M, Xu G, Wang H. Application of GFR-estimating equations in Chinese patients with chronic kidney disease. American journal of kidney diseases 45(3): 463-472, 2005.
25. Hallan S, Asberg A, Lindberg M, Johnsen H. Validation of the modification of diet in renal disease formula for estimating GFR with special emphasis on calibration of the serum creatinine assay. American journal of kidney diseases 44(1): 84-93, 2004.
26. Rule AD, Gussak HM, Pond GR, Bergstralh EJ, Stegall MD, Cosio FG, Larson TS. Measured and estimated GFR in healthy potential kidney donors. American journal of kidney diseases 43(1): 112-119, 2004.
27. Marx GM, Blake GM, Galani E, Steer BC, Harper SE, Adamson KL, Bailey DL, Harper PG. Evaluation of the Cockcroft-Gault, Jelliffe and Wright formula in

estimating renal function in elderly cancer patients. Annals of oncology 15: 291-295, 2004.
28. Lin J, Knight EL, Hogan ML, Singh AK. A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease. J Am Soc Nephrol 14: 2573-2580, 2003.
29. Bostom AG, Kronenberg F, Ritz E. Predictive performance of renal function equations for patients with chronic kidney disease and normal serum creatinine levels. J Am Soc Nephrol 13: 2140-2144, 2002.
30. Price CP, Finney H. Developments in the assessment of glomerular filtration rate. Clinica Chimica Acta 297: 55-66, 2000.
31. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function- a review. Clin Chem Lab Med 37(4): 389-395, 1999.
32. Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW. Biologic variation of cystatin C: implications for the assessment of glomerular filtration rate. Clinical chemistry 44(7): 1535-1539, 1998.
33. Randers E, Kristensen JH, Erlandsen EJ, Danielsen H. Serum cystatin C as a marker of renal function. Scand J Clin Lab Invest 58: 585-592, 1998.
34. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. American journal of kidney diseases 40(2): 221-226, 2002.
35. Vinge E, Lindergård B, Nilsson-Ehle P, Grubb A. Relationship among serum cystatin C, lean tissue mass and glomerular filtration rate in healthy adults. Scand J Clin Lab Invest 59: 587-592, 1999.
36. Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C (γ-trace) as a measure of the glomerular filtration rate. 45: 97-101, 1985.
37. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of the glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. Scand J Clin Lab Invest 64: 25-30, 2004.
38. Schück O, Teplan V, Stollová M, Skibová J. Estimation of glomerular filtration rate in obese patients with chronic renal impairment based on serum cystatin C levels. Clinical nephrology 62(2): 92-96, 2004.
39. Wasen E, Isoaho R, Matilla K, Valhberg T, Kivela SL, Irjala K. Estimation of glomerular filtration rate in the elderly: a comparison of the creatinine-based formulae with serum cystatin C. Journal of Internal Medicine 256: 70-78, 2004.
40. Hoek FJ, Kemperman FAW, Krediet RT. A comparison between Cystatin C, plasma creatinine and the Cockcroft-Gault formula for the estimation of glomerular filtration rate. Nephrol Dial Tranplant 18: 2024-2031, 2003.
41. Page MK, Bükki J, Luppa P, Neumeier D. Clinical value of cystatin C determination. Clinica Chimica Acta 297: 67-72, 2000.
42. Coll E, Botey A, Alvarez L, Poch E, Quintó L, Saurina A, Vera M, Piera C, Darnell A. Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. 36(1): 29-34, 2000.
43. Filler G, Bökenkamp A, Hofmann W, Le Bricon T, Martinez-Brú C, Grubb A. Cystatin C as a marker of GFR- History, indications, and future research. Clinical Biochemistry 38:1-8, 2005.

44. Laterza OF, Price CP, Scott MG. Cystatin C: An improved estimator of glomerular filtration rate. Clinical Chemistry 48(5): 699-707, 2002.
45. Randers E, Erlandsen EJ. Serum Cystatin C as an endogenous marker of the renal function- a review. Clin Chem Lab Med 37(4): 389-395, 1999.
46. Meijer AJ, Lamers WH, Chamuleau RAFM. Nitrogen metabolism and ornithine cycle function. Physiological Reviews 70(3): 701-748, 1990.
47. Bröchner-Mortensen J. A simple method for the determination of glomerular filtration rate. Scand J Clin Lab Invest 30: 271-274, 1972.
48. Bröchner-Mortensen J, Giese J, Rossing N. Renal Inulin Clearance versus total plasma clearance of 51Cr-EDTA. Scand J Clin Lab Invest 23: 301-305, 1969.
49. Rabito CA, Moore RH, Bougas C, Dragotakes SC. Noninvasive, Real-time monitoring of renal function: The ambulatory renal monitor. J Nucl Med 34: 199-207, 1993.