Chapter

Calculation of GFR via the Slope-Intercept Method in Nuclear Medicine

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Abstract

A determination of the glomerular filtration rate (GFR) with high accuracy is of great relevance especially in cases of insufficient kidney function. In nuclear medicine, the standard method is based on blood sample measurements with Cr-51 ethylenediaminetetraacetic acid (Cr-51-EDTA) or Tc-99m diethylene-triamine-pentaacetate (Tc-99m-DTPA), providing very high accuracy and reliability. In particular, the slope-intercept method turned out to be the most appropriate and is therefore routinely used in many hospitals worldwide. For this purpose, blood samples are drawn at certain time points starting 120 minutes after injection, which are then measured together with a standard probe in a gamma counter; based on the results, the GFR calculation is then usually performed automatically with an appropriate software. In this chapter, the mathematical background as well as a step-by-step description of the slope-intercept method is given. In our study, we found that at least three blood samples should be drawn in order to achieve highest quality and reliability. Furthermore, a sample size of at least three blood samples allows an error calculation which provides an estimation of the reliability of the preceding measurement.

Keywords: glomerular filtration rate, slope-intercept method, error calculation, nuclear medicine, Cr-EDTA, Tc-DTPA

1. Introduction

The glomerular filtration rate (GFR) is an important clinical measure for estimating not only the health of the kidneys but also the overall health of a patient, since it is directly proportional to the number of working nephrons. However, an exact determination of the GFR is not simple, and very often, an estimated GFR (eGFR) is calculated from the serum creatinine in the blood [1, 2]. For this, various formulas are available for different purposes [2–4]. Although these methods are convenient, they are not very sensitive, in particular in the case of insufficient kidney function [5].

An approved method for an accurate determination of the GFR was the inulin clearance, because inulin serves as a marker which is filtered by the glomeruli without tubular secretion or reabsorption [6]. Considered as gold standard, this method is time-consuming and needs urine as well as blood sample collection.

In nuclear medicine, several invasive and noninvasive methods are available to calculate the GFR. In principle, radiotracers, i.e., biological markers labeled with a radioactive isotope, are injected into the patient. The behavior of the tracer gives

information about the health condition of the organ and can be tracked by the emitted radiation from the labeled isotope. After injection, the radiation and therefore the concentration of the tracer can be measured either from drawn blood samples or with imaging techniques (so-called renal scintigraphy). The latter thus additionally allow a visualization of the anatomical properties of the organ.

To give an example, the very common radio tracer MAG3 (mercaptoacetyltriglycine, labeled to the gamma emitting isotope Tc-99m), providing excellent image quality, is not filtered in the glomeruli and therefore used with imaging techniques to determine the split renal function and the renal transit [7]. In contrary, the tracers Tc-99m diethylene-triamine-pentaacetate (Tc-99m-DTPA) and Cr-51 ethylenediaminetetraacetic acid (Cr-51-EDTA) are similar to inulin and therefore used to determine the GFR [8].

Cr-51-EDTA is only suitable for blood sample measurements since the physical properties of its labeled isotope Cr-51 do not allow the usage of imaging techniques. Tc-99m-DTPA on the other hand might be used for both blood sample and imaging methods.

For the sake of completeness, it is mentioned that methods are available to estimate the GFR from renal scintigraphy images, based on the accumulation of Tc-99m-DTPA in the kidneys within the first minutes after injection [9]. However, these methods only provide an estimation of the GFR and will therefore not be discussed in this chapter.

The main purpose of this chapter is to introduce the idea and the measurement procedure of the so-called slope-intercept method. In short, an appropriate tracer such as Tc-99m-DTPA or Cr-51-EDTA is injected, and at least two blood samples are taken at certain time points after the injections. The blood samples are then measured in a detector, a so-called gamma counter, in order to determine the emitted radiation, from which the GFR can be calculated. Methods using only one or two blood samples exist, but these are less accurate and error-prone [10]. The most accurate method involves the measurement of at least three blood samples because in this case a determination of the systematic error can be provided which in turn gives information about the reliability of the measurement.

2. Mathematical background

2.1 GFR calculation

For the determination of the GFR from an appropriate tracer, e.g., Tc-99m-DTPA or Cr-51-EDTA, the area under the so-called plasma concentration curve is needed, which is obtained from the drawn blood samples as described in the following.

After injection, the tracer travels through the blood vessels into the kidneys, where it is freely filtered and finally excreted. Assuming that other renal processes of the tracer are negligible, the decrease of the tracer concentration in the blood plasma after certain time points is then a measure for the glomerular filtration. Ideally, starting at 1 hour after injection, every 30 or 60 minutes a blood sample is drawn, in particular in the case of three blood samples at 120, 180, and 240 minutes after injection (see **Figure 1**) [11]. Due to its radioactivity, i.e., its emission of radiation, the tracer concentration in the blood plasma samples can be measured, usually with a gamma counter which allows the measurement of small samples.

The decrease of the tracer concentration in the blood plasma, expressed with a function $P(t)$, follows an exponential decay. This means due to glomerular filtration, the initial tracer concentration in the body (P_0) is decreasing exponentially (time, t) with a certain biological decrease constant L.

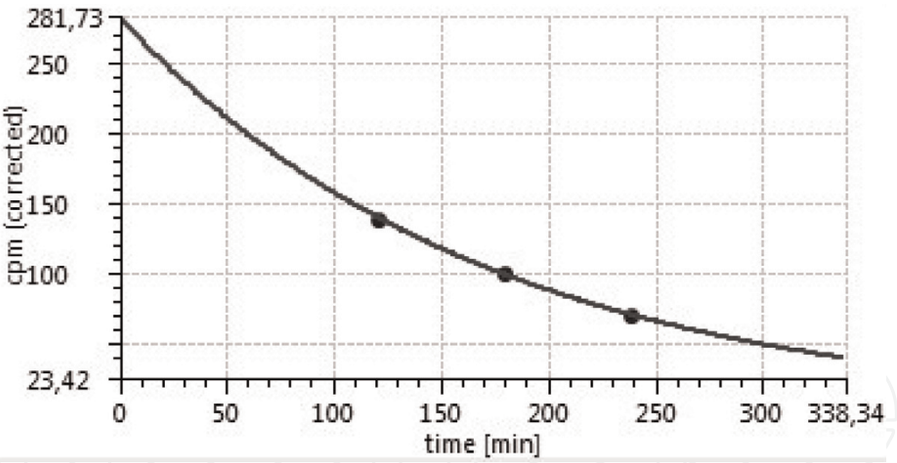

Figure 1.
Measured tracer concentrations of three plasma samples are plotted as dots; an exponential curve (see Eq. (1)) was fitted through the data points (line). The abscissa gives the time in minutes after injection of the tracer.

$$P(t) = P_0 \cdot e^{-L \cdot t} \tag{1}$$

The area A under this curve obviously is

$$A = \frac{P_0}{L} \tag{2}$$

The curve $P(t)$ is obtained by fitting the measured blood plasma samples with the exponential function from Eq. (1) (see **Figure 1**), e.g., with a least squares algorithm. The fit parameters are the values P_0 (the "intercept") and L (the "slope"), which are then used to calculate A.

Another information needed for the GFR calculation is the applied dose to the patient. While the syringe with the tracer is measured in an activimeter (or a comparable detector) before injection, the blood samples, showing considerably less radioactivity, are measured in a very different device (gamma counter). In order to connect the measurements of both devices, a so-called standard (a small amount of the tracer) must be prepared, which is then measured in both devices. The ratio of these two measurements is used to convert the injected dose measured in the activimeter to the units of the gamma counter. The converted applied dose D therefore can be written as

$$D = Act_{Syringe} \cdot \frac{Act_{Std}}{GC_{Std}} \tag{3}$$

with $Act_{Syringe}$ as measured syringe activity before application and Act_{Std} as standard activity in the activimeter and GC_{Std} as measured standard activity in the gamma counter.

The GFR can then be expressed as the converted total dose applied to the patient, D, divided by the area under the plasma concentration curve [11]

$$GFR = \frac{D \cdot V}{A} \tag{4}$$

with V as the dilution of the standard (usually around 500, see Section 3). Therefore, using Eqs. (2) and (3), the GFR can be written as

$$GFR = Act_{Syringe} \cdot \frac{Act_{Std}}{GC_{Std}} \cdot V \cdot \frac{L}{P_0} \tag{5}$$

2.2 Corrections

2.2.1 AUC correction

Due to underlying biological processes, the plasma concentration curve $P(t)$ appears not as a perfect exponential decay in particular in the beginning, leading to a wrong area under the curve (AUC). This can be solved by fitting the curve with multiple exponential curves, which would need much more blood samples. Another option is to start blood sample withdrawal after 120 minutes, i.e., after initial renal processes, using a simple AUC correction formula. Several formulas for adults and children are provided [11–14]. The Brochner-Mortensen correction is recommended [11, 15]:

For adults

$$GFR_{corr} = 0.9908 \cdot GFR - 0.001218 \cdot GFR^2$$

For children

$$GFR_{corr} = 1.01 \cdot GFR - 0.0017 \cdot GFR^2$$

2.2.2 Radioactive decay correction

Furthermore, both tracers not only have a biological half-life due to their glomerular clearance but also a physical half-life due to the radioactivity of their labeled isotopes. Consequently, the tracer concentration in the blood samples not only decreases due to the biological clearance but virtually also due to the physical loss of decayed isotopes which are labeled to the tracer.

Keeping in mind that the blood samples for the GFR determination with the slope-intercept method must be drawn 3 or even more hours after injection and the half-life of the isotope is not infinite, the physical half-life of the isotope might lead to a significant loss of tracer concentration due to its radioactivity (and not due to glomerular filtration). Ideally, the physical half-life of the corresponding isotope therefore should be very high in order to minimize the concentration loss due to radioactivity. This issue is illustrated in **Figure 2**.

In case of Cr-51-EDTA, the physical half-life of Cr-51 is 27.7 days. Assuming that, starting with injection, the blood sample withdrawing takes 4 hours, one can easily calculate that the virtual loss of tracer concentration due to its radioactivity during this time interval is about 1%. The radioactivity of Cr-51 therefore can be considered as negligible and the tracer can be treated as physically stable (see **Figure 2**).

On the other hand, the isotope Tc-99m from the tracer Tc-99m-DTPA has a physical half-life of about 6 hours; the concentration loss during a time period of 4 hours is not negligible anymore. As illustrated in **Figure 2**, measured values of drawn sample appear with a significantly lower measured concentration value due to the radioactive decay of Tc-99m; in this example, the calculated GFR would be falsely overstated by 20%. Thus, measurements with Tc-99m-DTPA must be corrected for the physical half-life of Tc-99m.

2.2.3 Background correction

Another important issue is the unavoidable measurement of unintended radioactivity. First, the remaining radioactivity in the syringe after injection must be measured and subtracted from the applied dose. Furthermore, both activimeter and

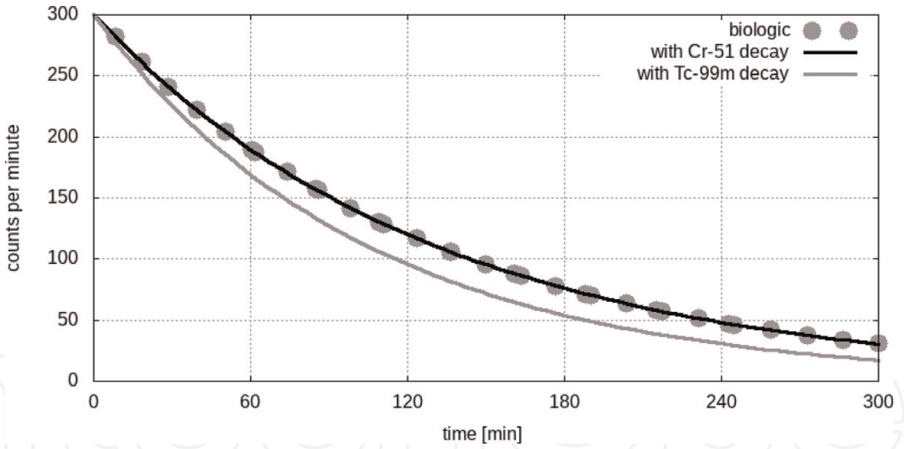

Figure 2.
Exponential decrease due to glomerular filtration with a typical half-life of 90 minutes (gray dots). Due to radioactive decay, real obtained curves are shown as black line in the case of Cr-51 and as gray line in the case of Tc-99m.

gamma counter continuously measure background radiation which must be subtracted from all measured values.

2.2.4 BSA correction

Since the GFR varies with the body surface area (BSA), it usually is presented as pure value but also corrected for the body surface area (BSA-GFR), normalized to the "standard man" (body surface 1.73 m^2). Several formalisms are available to estimate the body surface area for adults and children [16–20].

2.3 Error calculation

In clinical routine, irregularities during the measurement process, inadvertence, radioactive contamination, and other so-called systematic errors might lead to inaccurate results. Statistical (random) errors from the activimeter and gamma counter measurements are assumed to be negligible [10].

To estimate these systematic errors, the error of the area under the tracer concentration curve A (Eq. (2)) can be calculated after the fitting procedure [10], provided at least three blood samples have been taken. According to the Gaussian error propagation law, the errors of L and P_0 need to be calculated in order to obtain the error of A:

$$s_A = \sqrt{\left(\frac{s_{P0}}{L}\right)^2 + \left(\frac{s_L \cdot P_0}{L^2}\right)^2} \tag{6}$$

with s_A as error of the area under the curve A, s_{P0} as error of P_0, and s_L as error of L. This problem can be solved analytically, leading to

$$s_L = \sqrt{n \cdot \frac{1}{n-2} \cdot \frac{\sum \left(\ln P_0 - Lt_i - lnP(t_i)\right)^2}{n\sum t_i^2 - \left(\sum t_i\right)^2}} \tag{7}$$

$$s_{P0} = P_0 \cdot \sqrt{\sum {t_i}^2 \cdot \frac{1}{n-2} \cdot \frac{\sum \left(\ln P_0 - Lt_i - lnP(t_i)\right)^2}{n\sum t_i^2 - \left(\sum t_i\right)^2}} \tag{8}$$

with t_i as time interval after injection.

Under the assumption that random errors are negligible, s_A represents the error of the calculated GFR. Although errors of <10% are considered insignificant, high errors allow to identify irregularities and re-evaluate the results.

3. Measurement

3.1 Preparation

A standard is prepared, i.e., 1 ml of the used tracer is filled in an appropriate holder. Both the standard and the full syringe are measured in the activimeter. The empty syringe must also be measured after application in order to subtract the remaining activity from the measured value before application. In Eq. (3), this delivers the values $Act_{Syringe}$ and Act_{Std}.

3.2 Blood sample measurement

At least three blood samples are taken, starting at 120 minutes after injection, with an interval of 1 hour. The exact time period between injection and blood sample withdrawal needs to be recorded in order to minimize errors.

The standard usually needs to be diluted, e.g., by a factor of around 500. Around 1 ml is transferred into holders appropriate for the gamma counter. After separating blood plasma from hematocrit, 1 ml of each plasma sample is also transferred into holders for the gamma counter; all probes are measured. This gives the value GC_{Std} as well as all necessary data points to obtain the plasma concentration curve $P(t)$ (Eq. (1)).

3.3 GFR calculation procedure

Before starting any calculations, all measured values need to be corrected for background. Note that in case of Tc-99m-DTPA, values need to be corrected for radioactive decay. The measured data points are fitted with an exponential function (Eq. (1)) to obtain the plasma concentration curve function $P(t)$ and from this P_0 and L, which are needed to calculate the GFR (Eq. (5)).

If possible, i.e., if more than two blood samples have been drawn, the error is calculated according to Eq. (6). Furthermore, AUC correction and BSA correction are applied to the final GFR result.

4. Comparison of different blood sample methods

There are several methods allowing an estimation of the GFR from only one blood sample [21–24]. Although these methods are from high convenience for the routine and the patients, they are not recommended for low GFRs [11] and show significant deviations to the slope-intercept method [10].

The slope-intercept method with blood samples drawn after 120 minutes after injection is suggested to be the best compromise between accuracy and simplicity; it furthermore is a repeatable and reliable method [10, 11, 25, 26]. Since an error calculation helps in identifying errors in the measurement process such as radioactive contamination or irregularities in the routine, the slope-intercept method with three blood samples appears ideal.

5. Conclusions

The slope-intercept method is based on several blood samples and an accurate calculation procedure including corrections and error estimation. In particular in case of low GFRs, this method is the best compromise between the effort for the clinical routine, patients comfort, and accuracy.

Appendices and nomenclature

Abbreviations

AUC	area under the curve
BSA	body surface area
GFR	glomerular filtration rate

Nomenclature

Tc-99m-DTPA	Tc-99m diethylene-triamine-pentaacetate
Cr-51-EDTA	Cr-51ethylenediaminetetraacetic acid

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