A SAS PROCEDURE FOR EVALUATING INULIN CLEARANCE DATA

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Abstract

In clinical nephrology it is of considerable importance to determine renal function as correctly as possible. For that purpose, however, there are only a few methods available. One of which is the so-called single shot inulin clearance. Up to now, this method has been too tiresome to be applied routinely. Since the arrival of a new test kit for inulin, this clearance technique is more widely used. Now the more tiresome part is the calculation of the clearance itself. For that purpose we have written a SAS procedure based on a two compartment model. We describe two approaches to the problem: one with and one without using SAS Graph. A general flow chart of the procedure reads as follows: reading in the data; regression analysis of the latest part of the clearance data and output of the regression measures; regression analysis of the earlier part of the clearance data and output of the regression measures; determination of the clearance by calculating the area under the approximated curve; examination of adequacy of the line fitting by checking the graphical output and regression measures; decision on adequacy of the line fitting; re-running of the program, if necessary; graphical and written output. The procedure may be used either interactively or as a batch procedure. It allows the analysis of the inulin clearance on any type of computer used in the routine laboratory, as long as SAS is installed. Furthermore, it may be used for any other single-shot-based clearance method.

Introduction

An adequate determination of renal function is becoming more and more important, as new therapies claim to prevent the progression of chronic renal failure into endstage renal failure. Thus, an adequate monitoring of these treatment modalities is mandatory. There are, however, only a few methods available for the correct assessment of renal failure. One approach is by isotope methods and one by inulin. Both approaches use the so-called single shot clearance. Up to now, the chemical determination of inulin has been very tiresome and thus could not be applied routinely. Since the arrival of a new test kit for the inulin determination this clearance technique is more widely used (figure 1). Today, the more tiresome part is the calculation of the clearance itself.

Thus, the aim of this paper is to describe a program for the calculation of a single shot plasma clearance (appendix A and B). The calculation is based on a two compartment model. It may be used either on a mainframe or on a PC. For the PC version also a procedure is described for the use without having SAS/GRAPH installed.

Description of the procedure

After the injection inulin exhibits a rapid rise in its plasma concentration followed by a nonlinear rapid decrease and a subsequent
linear fall. The program is based on a two compartment model. Thus two regression lines have to be adjusted to the curve. Thereafter the area under these lines/curve is to be determined. This area is then converted into the respective clearance.

In a first data step, the time of blood sampling in minutes and the concentration of inulin is read in (the raw data are given in Appendix A). Then the time is converted into hours and the inulin concentrations are converted into logarithms. The data may be either written directly into the program or may be kept, for example, in a separate dBase data set. It may be more convenient, however, to store them with the program, as often the program will be run interactively, and especially as body weight and height have to be added at the end of the calculation.

In a next step, the data are divided into two parts. One including the steep decrease, which ends roughly after the first hour, the other contains the much slower, linear fall. As cut-off point we use 1 hour. Then a regression analysis is performed on the data of the linear part. The cut-off point, however, has to be changed, if the data are not linear. The linearity has to be estimated from the respective graphical output (figure 2 and 3) and the regression analysis. The results of such a regression analysis are given in Appendix C. It is of note that the intercept is still logarithmic, while the slope is linear. Furthermore, every time such an analysis is performed it is warranted to check the adequacy of the regression line adjusted by examining $R^2$. The same is true for the next regression analysis performed on the steeply decreasing part.

In the next data step, the parameters for the slope and the intercept of the calculated linear regression are read in. Thereafter, multiple data points for this regression line are generated.

In the next data step, the data of the regression line are merged with the raw data. This is done for the data of the steep decrease, i.e. time less than 1 hour. This is necessary as here the contribution of the steep decrease to the clearance has to be determined by calculating the difference between the slope of regression line and the actual concentrations. Thereafter, the logarithms are taken from these differences. Caution: Here an error message may occur, if negative differences arise. This most often occurs when the clearance is very low. Thereafter, a second regression analysis on the steep data is performed. Again the adequacy of the calculated regression line should be checked in the graphical output and by assessing $R^2$. Caution: Here again an error message may arise when the number of data points had been reduced to less than two, which may be due to a negative difference between the actual values and the data of the regression line as described in this data step. Thus it is not possible to perform a regression analysis. Despite that missing second regression line it is possible to calculate the area under the curve and thus the inulin clearance. The occurring error in the calculation is minute. Under the described circumstances, the calculation of the inulin clearance is only based on the linear part of the data. This occurs only in patients with poor renal function. Other problems, however, should not occur with this program.

In a next data step, the points on the regression line of the second regression analysis are produced.

Finally, the raw data and the data of the two regression lines are merged. Then these data are plotted. For a PC without SAS/GRAPH we recommend to represent the different lines by either 1s or 2s, and the raw data by zero (figure 2). Thus, outliers can easily be detected visually. Concerning a plot with SAS/GRAPH, the option I=join and v=none should be used. When the program is used with a plotter or a video screen colours should be used.
In the final data step, only the data points of the two regression lines are used in order to calculate the area under the curve. In this data step, the dosis of inulin, body weight and height have to be added. The clearance is calculated as inulin dosis divided by the area under the curve multiplied by 60 in order to convert the hours back into minutes. Normally the inulin clearance is given according to body surface. The body surface is calculated according to the formula of Bois-Bois. Thereafter, the uncorrected inulin clearance is standardized either on m² as in pediatrics or on 1.73 m² as in internal medicine. Then, these data are printed (figure 1, last line).

**Flow sheet of the procedure**

- reading in of the raw data
- regression analysis on the linear part of the data
- creating data points on this regression line in 0.01 intervals
- regression analysis on the difference of the linear and the nonlinear part of the data
- creating data points on this regression line in 0.01 intervals
- plot of the raw data and the two regression lines
- calculation of the area under the curve and the inulin clearance, correction for body surface

**Clinical applicability**

The presented procedure is suitable for any clearance method based on a single shot approach. This holds true for any procedure in which a two compartment model can be assumed. Thus, beside inulin it may be also suitable for any other isotopic method. In addition to the inulin clearance, data on intravascular and extracellular space can be obtained during the performance of an inulin clearance. The extracellular space can be calculated from the inulin concentration at 120 min., the initial dose given and the total amount of inulin excreted up to that point. Furthermore, it is possible to estimate the intravascular space from the inulin concentration in blood determined during the first five minutes after the application. Overall this procedure guarantees a fast and easy to apply approach to calculation of a single shot clearance.

**Acknowledgements**

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References


Appendix A
Calculation of the inulin clearance without using SAS-Graph.

data a;
input time c ;
*time=time of blood sampling (min);
* c=inulin concentration;
th=time/60; t=round(th,.01);
lnc=log(c); p=0;
cards;
10 0.782
20 0.620
30 0.550
40 0.499
120 0.328
150 0.283
180 0.264
210 0.267
240 0.216
;proc sort; by t;

data b; set a; if t>0.99;
proc reg outest=c; model lnc=t; run;

data d; set c; p=1;
a1=t; b1=intercep;
do t=0 to 4 by 0.01; t=round(t,.01);
y1=a1*t+b1; c1=exp(y1);
keep t p a1 b1 y1 c1; output; end; run;

data e; merge a d; by t; if t>0.99 then delete;
diffc=c-c1; lnc2=log(diffc); if lnc2=. then delete;
keep t lnc2; run;
proc reg outest=f; model lnc2=t; run;

data g;set f;p=2;
a2=t; b2=intercep;
do t=0 to 4 by 0.1; t=round(t,.01);
y2=a2*t+b2; c2=exp(y2);
keep p a2 b2 y2 t c2; output; end; run;

data plot; merge a d g; by p;
proc plot; plot (y1 y2 lnc)*t=p /overlay
   vaxis=-2 to 0 by 0.4 haxis=0 to 4 by .5; run;

data area; merge d g; by t;if t=0;
area=((c1/a1)+(c2/a2))*(-1); *area under the curve;
dosis=5000; *inulin dosage;
Cin=dosis/(area*60); *uncorrected inulin clearance (ml/min);
height=170; *body height (cm);
weight=70; *body weight (kg);
surface=0.007184*(weight**0.425)*(height**0.725); *body surface (Bois-Bois-formula);
Cincor=Cin/surface; *body surface corrected Cin(ml/min/m2);

proc print;run;
Calculation of the inulin clearance when using SAS-Graph.

```sas
options nocenter errors=2 ps=42;
input time c;
  th=time/60; t=round(th,.01);
  Inc=log(c); p=0;
cards;
  1  0.01
  20 0.620
  30 0.550
  40 0.499
  120 0.328
  150 0.283
  180 0.264
  210 0.267
  240 0.216
;proc sort; by t;
data b; set a; if t>0.99;
  proc reg noprint outest=c; model Inc=t; run;
data d; set c; p=1;
  a1=t; b1=intercep;
  do t=0 to 4 by 0.01; t=round(t,.01);
    y1=a1*t+b1; c1=exp(y1);
  keep t p a1 b1 y1 c1; output; end; run;
data e; merge a d; by t; if t>0.99 then delete;
  diffc=c-c1; Inc2=log(diffc); if Inc2=. then delete;
  keep t Inc2; run;
  proc reg noprint outest=f; model Inc2=ti runi;
data g;set f;p=2;
  a2=t; b2=intercep;
  do t=0 to 4 by 0.1; t=round(t,.01);
    y2=a2*t+b2; c2=exp(y2);
  keep p a2 b2 y2 t c2; output; end; run;
data plot; merge a d g; by p;
  goptions hpos=30 vpos=30 hsize=9 vsize=9;
  proc gplot; plot (y1 y2 Inc)*t /overlay
    vaxis=-2 to 0 by 0.4 haxis=0 to 4.0 by 1;
  symbol1 i=join v=none l=2 w=4 c=red;
  symbol2 i=join v=none l=3 w=4 c=green;
  symbol3 i=JOIN v=star l=1 w=4 c=white; run;
data area; merge d g; by t;if t=0;
  area=((c1/a1)+(c2/a2))*(-1); *area under the curve;
  dosis=5000; *inulin dosage;
  Cin=dosis/(area*60); *uncorrected inulin clearance (ml/min);
  height=170; *body height (cm);
  weight=70; *body weight (kg);
  surface=0.007184*(weight**0.425)*(height**0.725); *body surface (Bois-Bois-formula);
  Cincor=Cin/surface; *body surface corrected Cin(ml/min/m2);
proc print;run;
```
Appendix C

Output of the regression analysis of the two regression lines adjusted to the different parts of the two compartment model. SAS-Graph.

Dep Variable: LNC

First/major regression line

### Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1</td>
<td>0.07986</td>
<td>0.07986</td>
<td>22.625</td>
<td>0.0176</td>
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<tr>
<td>Error</td>
<td>3</td>
<td>0.01059</td>
<td>0.00353</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Total</td>
<td>4</td>
<td>0.09045</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Root MSE 0.05941
Dep Mean -1.31237
C.V. -4.52717

### Root MSE 0.05941

R-Square 0.8829
Dep Mean Adj R-Sq 0.8439

### Parameter Estimates

| Variable  | DF | Parameter Estimate | Standard Error | T for HO: Parameter=0 | Prob > |T| |
|-----------|----|-------------------|----------------|------------------------|--------|
| INTERCEP  | 1  | -0.776177         | 0.11581753     | -6.702                 | 0.0068 |
| T         | 1  | -0.178734         | 0.03757617     | -4.757                 | 0.0176 |

### Dep Variable: LNC2

Second regression line

### Analysis of Variance

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<th>Source</th>
<th>DF</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>0.91580</td>
<td>0.91580</td>
<td>91.801</td>
<td>0.0107</td>
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<tr>
<td>Error</td>
<td>2</td>
<td>0.01995</td>
<td>0.00998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Total</td>
<td>3</td>
<td>0.93575</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Root MSE 0.09988
Dep Mean -1.80472
C.V. -5.53437

### Parameter Estimates

| Variable  | DF | Parameter Estimate | Standard Error | T for HO: Parameter=0 | Prob > |T| |
|-----------|----|-------------------|----------------|------------------------|--------|
| INTERCEP  | 1  | -0.734901         | 0.12231619     | -6.008                 | 0.0266 |
| T         | 1  | -2.562431         | 0.26744165     | -9.581                 | 0.0107 |
**Figure 1**

Currently in our hospital used scheme for blood and urine sampling when performing an inulin clearance.

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood Sampling</th>
<th>Urine Sampling</th>
<th>Urine Volume</th>
</tr>
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<tbody>
<tr>
<td>0 min:</td>
<td>0 min:</td>
<td>0 min:</td>
<td>ml</td>
</tr>
<tr>
<td>15 min:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min:</td>
<td>30 min:</td>
<td></td>
<td>ml</td>
</tr>
<tr>
<td>45 min:</td>
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<tr>
<td>60 min:</td>
<td>60 min:</td>
<td></td>
<td>ml</td>
</tr>
<tr>
<td>90 min:</td>
<td>90 min:</td>
<td></td>
<td>ml</td>
</tr>
<tr>
<td>120 min:</td>
<td></td>
<td>150 min:</td>
<td>ml</td>
</tr>
<tr>
<td>180 min:</td>
<td></td>
<td>210 min:</td>
<td>ml</td>
</tr>
<tr>
<td>240 min:</td>
<td></td>
<td>270 min:</td>
<td>ml</td>
</tr>
</tbody>
</table>

- Please give the exact time of the sampling, e.g. 10.30. Then deviations from the protocol are not that important.
- 2ml blood per sample are needed.
- 20ml of Inutest™ have to be injected, irrespective of the renal function.
- The emptying of the bladder is controlled by ultrasound.
**Figure 2**

"Graphical" output when not using SAS-Graph

Plot of $Y_1^T$ Symbol is value of $P$
Plot of $Y_2^T$ Symbol is value of $P$
Plot of $LNC^T$ Symbol is value of $P$

```
OBS  T  P   A1  B1  Y1  C1  A2  B2  Y2
1  0  2 -0.178734 -0.776167 -0.776167 0.460167 -2.56243 -0.734901 -0.734901

OBS  C2  AREA  DOSIS  CIN  HEIGHT  WEIGHT  SURFACE  CINCOR
1  0.479553  2.76174  5000  30.1742  170   70   1.80971  16.6735
```
Figure 3

Graphical output when using SAS-Graph