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Evaluation of the formula clearance as a measure of the glomerular filtration rate in cyclosporin-treated children following renal transplantation

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Abstract. Since the introduction of the nephrotoxic drug, cyclosporin, as the main immunosuppressive agent following organ transplantation, the need for an accurate method to determine the glomerular filtration rate (GFR) has arisen. In the present study the clearance of inulin has been compared to the clearance of creatinine estimated by short-term urine sampling or by the formula clearance in 29 children following renal transplantation. The children, 0.4–15.4 years of age at transplantation, were examined within 5 months following transplantation, and thereafter yearly. During the first 2 years after transplantation there was poor agreement between inulin clearance and formula clearance, while 3–4 years after transplantation there was good correlation between the two methods. However, the formula clearance generally overestimated GFR and the overestimation increased with decreasing renal function. In spite of the good correlations between the two methods after the first years following transplantation, the formula clearance in individual patients does not follow the inulin clearance changes very closely. In conclusion, formula clearance was found to be an inaccurate method of following GFR, especially during the first 2 years after transplantation. This might be caused by changes in habitus as well as an increased creatinine secretion caused by cyclosporin.

Key words: Glomerular filtration rate, in children – Creatinine clearance, in children – Children, renal function after transplantation

Introduction

Inulin clearance has long been considered the standard for measurements of the glomerular filtration rate (GFR) [10, 14, 15]. However, in several studies in renal transplant recipients GFR is measured by the clearance of creatinine with urine sampling [5, 11] or by the formula clearance [4, 9]. The formula clearance is calculated from the serum creatinine concentration and height of the patient according to Schwartz et al. [13] or Morris et al. [8]. Since cyclosporin A (CsA) was introduced as an immunosuppressive agent following transplantation, some authors claim that there is a hypersecretion of creatinine in the renal tubules leading to (1) an overestimation of GFR by the clearance of creatinine and (2) the opinion that the serum creatinine concentration does not accurately reflect GFR [12, 16]. The aim of the present investigation was, therefore, to analyse further the accuracy of estimating GFR by means of the formula clearance and the clearance of creatinine with short-term urine sampling, and to compare these methods with the clearance of inulin in a group of cyclosporin-treated children following kidney transplantation.

Material and methods

The children, 14 boys and 15 girls, 0.4–15.4 years old (median 7.1 years) at transplantation, were followed regularly with renal function tests. The follow-up time ranged from 0.2 to 5.7 (median 1.5) years. The diagnoses of the children are given in Table 1. Twenty children received their kidney from living related donors (LD) and 9 from cadaveric donors (CD). Nine of the children were treated with CsA and low-dose prednisolone and 20 had triple therapy including azathioprine. The immunosuppressive regimens during the two treatment periods are given in Table 2. Hypertension was being treated in 22 of the patients when their first renal function test was performed. A more thorough investigation of the long-term renal function in children following renal transplantation will be reported elsewhere (U. Berg, A. B. Bohlin, in preparation).

Renal function tests were performed within 5 months after transplantation and thereafter yearly. Renal function was evaluated as GFR tested by the clearance of inulin and by the formula clearance

Table 1. The underlying renal disorders in the 29 children followed up after renal transplantation

Diagnoses	No. of children
Renal dysplasia/agenesis	11
Juvenile nephronophtisis	6
Polycystic kidney disease	5
Glomerulonephritis	5
Other	2
Total	29

Table 2. The two different immunosuppressive regimes used. When no administration procedure is given, the doses are given orally. CsA, Cyclosporin; Pred, prednisolone; Aza, azathioprine; MP, methylprednisolone

Day	CsA and low-dose Pred		Triple therapy		
	CsA (mg/kg/per day)	Pred (mg/day)	CsA (mg/kg/per day)	Aza (mg/kg/per day)	Pred (mg/day)
−2	15	30	15	2	30
−1	15	30	15	2	30
0	2.5 i.v. ×3	1.5 + 0.5 g MP i.v.	3.3 i.v. ×3	2 i.v.	100 + 0.5 g MP i.v.
+1	12	1.35 mg/kg/per day	20[a]	2	90
+2	12	1.20 mg/kg/per day	20[a]	2	80
+3	12	1.05 mg/kg/per day	20[a]	2	70
+4	12	0.90 mg/kg/per day	20[a]	2	60
+5	12	0.75 mg/kg/per day	20[a]	2	50
+6	10	0.60 mg/kg/per day	20[a]	2	40
+7	10	0.45 mg/kg/per day	20[a]	2	30
+8	10	20[a]	20[a]	2	20
+20	8	20[a]	20[a]	2	20
+22	8	15[a]	20[a]	2	20
+30	8	15[a]	20[a]	1	15
+50	6	15[a]	20[a]	1	15
+52	6	10[a]	20[a]	1	15
+60	6	10[a]	20[a]	1	10
+90	6	5[a]	20[a]	1	alt. day

[a] Administered in two doses per day, adjusted to trough blood levels around 300 ng/ml during the first month, 250 ng/ml during the second month and 150 ng/ml during the third month corresponding to the specific monoclonal RIA technique

according to Morris et al. [8]. Altogether, 85 renal function tests were performed. Clearance of inulin was determined by a standard clearance technique including a continuous infusion after a prime dose. Water diuresis was induced and enabled the patients to empty their bladders by spontaneous voiding [1]. Four 30-min urine samples were collected and a blood sample was drawn midway through each urine period. The clearance values presented are the means of four clearance periods. Formula clearance was calculated from the serum creatinine concentration on the morning of the day when the inulin clearance was performed. Clearance of creatinine was determined in 20 patients from a timed short-term (about 4 h) morning urine sample before the inulin clearance investigation.

Inulin was analysed by the anthrone method [3] and creatinine was determined by a kinetic method based on a modified Jaffé reaction [6]. For the statistical analysis Students' *t*-test, the paired *t*-test, and the least-squares method for calculating the correlation and regression were used.

Results

Figure 1 a and b shows the relationship between the formula clearance and C inulin in the children studied within 5 months and 3 years following transplantation. Wide variations around the line of identity were found during the first 2 years and the regression line does not follow the line of identity. There was, however, a significant correlation with a coefficient of 0.543 within 5 months, but a formula clearance value of about 60 might correspond to a C inulin value ranging from 45 to 125 ml/min/1.73 m^2. One and two years after transplantation there was better agreement ($r = 0.754$, $n = 20$ and $r = 0.721$, $n = 15$, respectively) but most values are found above the line of identity. After 3 years (Fig. 1 B) and 4 years ($r = 0.887$, $n = 6$, $P < 0.05$) the agreement between the two methods seems better and the regression lines are close to the identity line.

Figure 2 shows the formula clearance/C-inulin ratio in relation to C inulin within 5 months after transplantation. There was a significant inverse correlation between the ratio formula clearance/C-inulin and C inulin. If there were total agreement between the two methods, the ratio should have been 1. Two thirds of the values fell above a ratio of 1 and, furthermore, the ratio increases with decreasing GFR. Figure 3 shows the C creatinine values after collection periods of about 4 h in relation to C inulin. There is a significant correlation, but generally C creatinine overestimates the GFR.

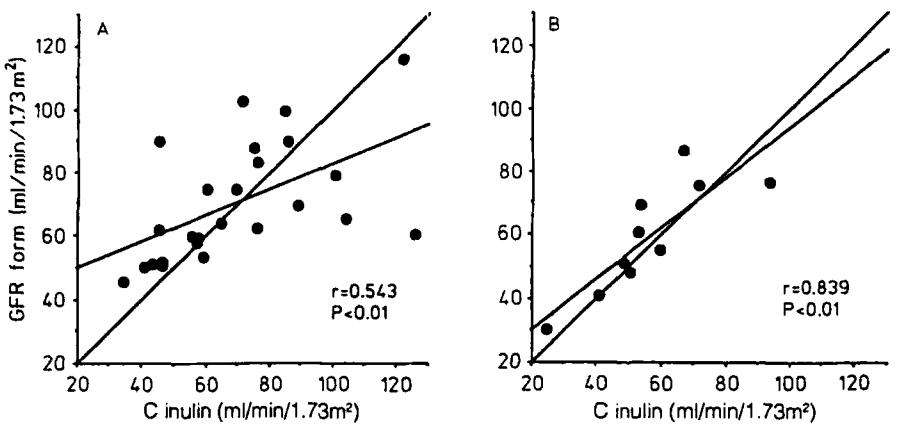

Fig. 1 A, B. The formula clearance *(GFR form)* in relation to inulin clearance *(C inulin)* determined simultaneously within **(A)** 5 months and **(B)** at 3 years after transplantation. The lines of identity as well as the regression lines are shown and also the correlation coefficients *(r)* and the significance levels *(P)*

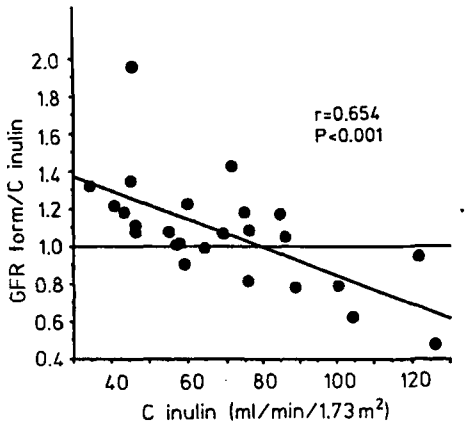

Fig. 2. The relation between the ratio of formula clearance to inulin clearance *(GFR-form/C-inulin)* and the inulin clearance in all patients studied within 5 months following transplantation. The regression line as well as the line where the ratio is 1 are plotted. The correlation coefficient *(r)* and the significant level *(P)* are given

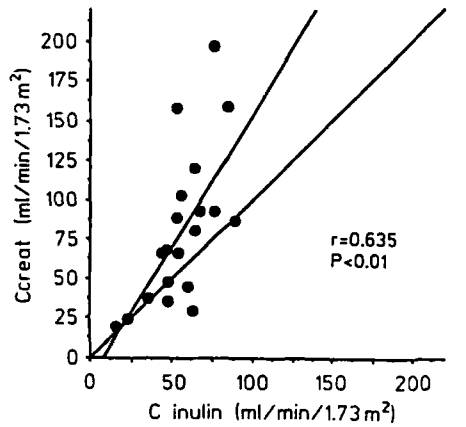

Fig. 3. The relationship between creatinine clearance and inulin clearance done on the same day in 20 patients. The line of identity and also the regression line are shown as well as the correlation coefficient *(r)* and the significance level *(P)*

According to Figure 1 B, i.e. with exclusion of the first 2 years following transplantation, there seemed to be rather good overall agreement between the formula clearance and C inulin in the group of patients. However, it is of great importance to determine whether the agreement persists when studying individual patients. In the following, some patients on whom more than two renal function tests were performed, are presented individually. Figures 4–6 show the formula clearance related to C inulin as well as GFR measured by the two methods during follow-up for 2–6 years in individual patients. Figure 4 A and b show the results in a child (patient 9) where the formula clearance clearly overestimated GFR with a mean formula clearance/C-inulin ratio of 1.36. Figure 5 A and B shows the results in a child (patient 3) with inverse correlation between the formula clearance and C inulin. The mean formula clearance/C-inulin ratio was 0.99. Figure 6a and b shows the GFR of a boy with a substantial muscle mass where the formula clearance underestimated GFR with a mean formula clearance/C-inulin ratio of 0.90. During follow-up the GFR sometimes changed in different directions with the two methods.

Discussion

The formula clearance according to Schwartz et al. [13] has been widely used and has been reported to give a good estimate of GFR in different renal disorders [2, 8, 13]. During the last few years it has also been used to estimate GFR in children following different organ transplantations [4, 7, 17]. Hoyer et al. demonstrated a significant correlation ($r = 0.85$) between formula clearance and inulin clearance, but with the regression line not following the line of identity [5]. The majority of their values were above the identity line, which indicates an overestimation of GFR by the formula clearance method. Furthermore, the regression analysis in their series was based on values obtained about 6 weeks after transplantation, a time when the great changes in body habitus have not yet appeared. The catch-up growth, in our own experience, generally starts when the steroid therapy has been changed to alternate day treatment about 3 months after transplantation. Furthermore, the increased appetite and consequent gain in weight, secondary to the steroids, might have just started when their clearance investigations were performed. The present study also demonstrated correlations between the formula clearance and C inulin but, especially during the first 2 years following transplantation when the greatest catch-up growth occurs (M. Englund, U. Berg, G. Tydin, in preparation), there were wide variations around the line of identity. Furthermore, the correlation coefficients found at

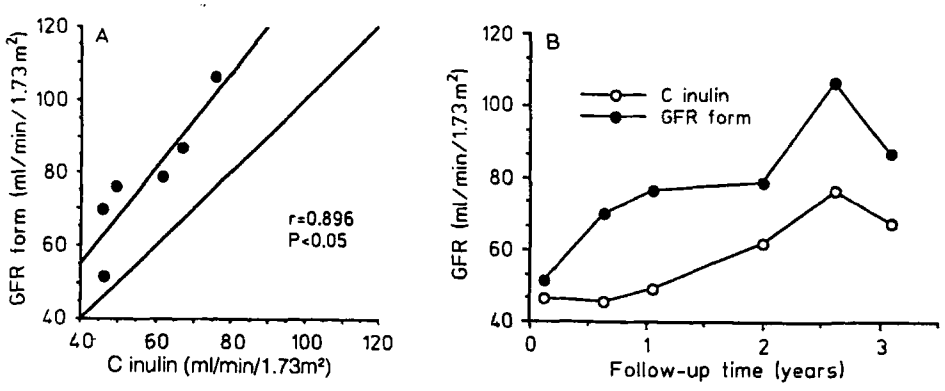

Fig. 4. A The formula clearance *(GFR form)* in relation to inulin clearance *(C inulin)* determined on different occasions after transplantation. The line of identity as well as the regression line are shown and also the correlation coefficient *(r)* and the significance level *(P)*. **B** The glomerular filtration rates determined by the two methods during follow-up. Results for patient 9 where the formula clearance clearly overestimated *GFR*

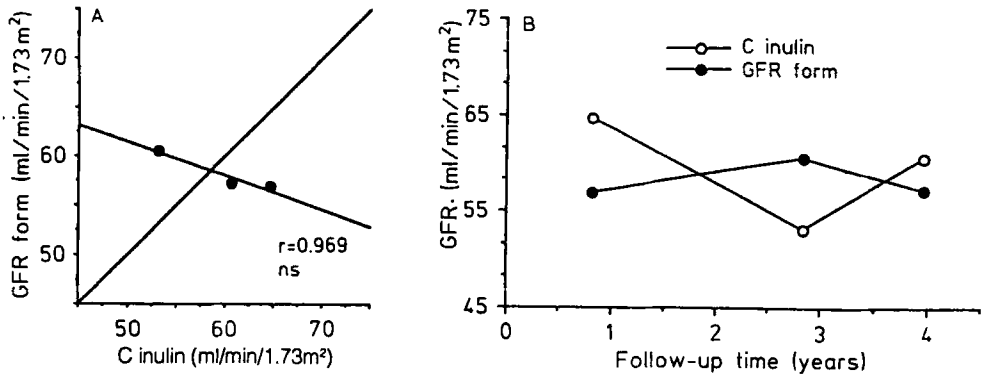

Fig. 5 A, B. As for Fig. 4. Results for patient 3 showing inverse correlation between the formula clearance *(GFR form)* and *C inulin*

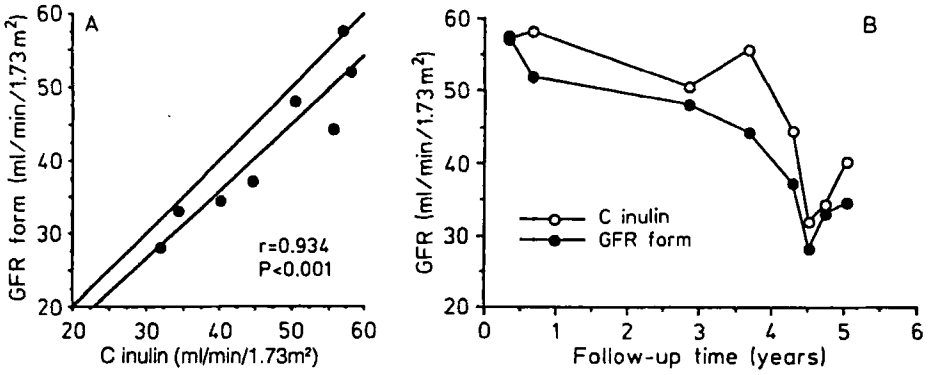

Fig. 6 A, B. As for Fig. 4. Results for patient 2 with substantial muscle mass where the formula clearance clearly underestimated *GFR*

< 5 months and at 1 and 2 years were much lower than those found after 3 and 4 years. Looking further at the follow-up of individual patients, the formula clearance both overestimated and underestimated C inulin. In some cases the formula clearance showed an inverse correlation with C inulin (Fig. 5) with mean formula clearance/C-inulin ratios about 1, which incorrectly seems to indicate a good agreement between the two methods. The constant underestimation of the formula clearance found in the well-trained boy with an adequate muscle mass (Fig. 6), and therefore a proportionately high serum creatinine level, shows why, in the whole group of patients, it seems as if the correlations between the two methods are extremely good.

Several factors might be responsible for the general overestimation of GFR by the formula clearance. Height is one factor which, because of the catch-up growth noted when alternate-day steroid therapy was instituted, might increase the formula clearance. The accuracy of the serum creatinine measurements in CsA-treated transplanted patients has also been discussed [12, 16]. The tubular dysfunction caused by CsA has been reported by several authors [2, 12, 16]. An increased proximal sodium reabsorption has been reported [2] as well as increased proximal tubular secretion of creatinine leading to increased creatinine clearance values and decreased serum creatinine concentrations [12, 16]. A low serum creatinine concentration might be a factor contributing to the overestimation of GFR by the formula. Furthermore, the discrepancy between the two methods seemed to increase with decreasing GFR, as shown by the inverse correlation between the formula clearance/C-inulin ratio and C inulin. This was also found by Tomlanovich et al. when looking at C-creatinine/C-inulin ratios against C inulin [16]. They suggested an increasing hypersecretion of creatinine with more advanced renal damage, which further blunts the elevation of serum creatinine and thereby obscures the decline of the true GFR.

However, formula clearance appears to be more accurate than clearance of creatinine with short-term collection periods (Fig. 3). This might be explained by inaccurate urine sampling as well as the above-mentioned hypersecretion increasing the urinary creatinine concentration and also decreasing the serum creatinine concentration.

The accuracy of inulin clearance might, of course, also be debated. The inulin molecule is rather large (5200 Da) and has been reported by Rosenbaum et al. [11] to be restricted in the glomerular filter. They studied their patients within 1 week after transplantation. However, these results could not be confirmed by Mak et al. who reported excellent agreement between the clearances of inulin, creatinine and non-radioactive iothalamate [5]. However, they reported, in agreement with the present investigation, that the estimated GFR by the formula clearance was significantly less precise than the standard clearance methods. The studies by Mak et al. [5] and Rosenbaum et al. [11] were probably made on transplanted patients not yet treated with CsA, which has to be taken into consideration when comparing their results with those reported here. Tomlanovich et al. conclude that in their CsA-induced chronic nephropathy, inulin is not restricted by the glomerular capillary wall and behaves like a true filtration marker [16].

In conclusion, despite a good overall correlation between formula clearance and inulin clearance in the group of patients, the formula clearance in individual cases can be quite inaccurate as a measure of the glomerular filtration rate, especially during the first years following trans-

plantation, when the habitus shows the greatest changes. Furthermore, the inaccuracy of formula clearance increases with decreasing renal function, giving falsely high values.

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