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# Serum cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy

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**Abstract.** Christensson AG, Grubb AO, Nilsson J-Å, Norrgren K, Sterner G, Sundkvist G (Malmö University Hospital, Malmö; and Lund University Hospital, Lund, Sweden). Serum cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. *J Intern Med* 2004; **256**: 510–518.

**Objective.** To determine whether serum cystatin C is more accurate than serum creatinine in the detection of diabetic nephropathy, also after adjustment for age. **Methods.** Forty-one patients with type 1 and 82 patients with type 2 diabetes were evaluated with serum creatinine, serum cystatin C, and <sup>51</sup>Cr-EDTA clearance (reference method). Cystatin C was measured by a particle-enhanced turbidimetric method and creatinine by an enzymatic method. Statistical estimations were performed both without and with age adjustment created by z-scores for <sup>51</sup>Cr-EDTA clearance, creatinine, and cystatin C. The cut-off levels for glomerular filtration rate (GFR) (<sup>51</sup>Cr-EDTA clearance) were 60 and 80 mL min<sup>-1</sup> 1.73 m<sup>-2</sup>, respectively, in absolute values and 80, 90 and 95% CIs, respectively, in age-adjusted values (z-scores).

**Results.** Estimations without age adjustment showed significantly ( $P = 0.0132$ ) closer correlation for cystatin C ( $r = 0.817$ ) versus <sup>51</sup>Cr-EDTA clearance as compared with creatinine ( $r = 0.678$ ). However, when using age-adjusted values, the correlation for cystatin C and creatinine, respectively, versus <sup>51</sup>Cr-EDTA clearance did not differ. When comparing the diagnostic utilities for serum cystatin C versus serum creatinine in manifest renal impairment ( $\text{GFR} < 60 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  or z-scores  $< -1.28$  SD), there were no significant differences between the two markers whether age adjusted or not. However, for diagnosing mild nephropathy ( $\text{GFR} < 80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  or z-score  $-0.84$  SD), serum cystatin C is significantly more useful. **Conclusions.** Serum cystatin C performed better compared with serum creatinine even when measured enzymatically, to detect mild diabetic nephropathy. However, serum creatinine was as efficient as serum cystatin C to detect advanced diabetic nephropathy.

**Keywords:** creatinine, cystatin C, diabetes, nephropathy, renal function.

## Introduction

The importance of detecting early impairment of renal function in patients with diabetes is evident [1]. Although, microalbuminuria is the first detectable functional abnormality, glomerular filtration rate (GFR) is the critical renal function [2]. GFR can be determined by different means. Measurement of

serum creatinine is simple but the general view is that up to 50% of GFR can be lost before significant elevation of serum creatinine occurs [3] also when specific enzymatic creatinine measurements are used [4]. In contrast to serum creatinine, inulin, Cr-EDTA or iothexol clearances are considered as the most accurate methods of determining GFR [5]. These methods are, however, expensive, complicated, and

time-consuming and thus not suitable for routine measurement. Therefore, it was a major step forward, when serum cystatin C, which is eliminated by glomerular filtration, reabsorbed, and catabolized in proximal renal tubular cells without tubular secretion, was introduced as an accurate endogenous GFR marker [6, 7]. Indeed, serum cystatin C was found superior to serum creatinine as a GFR marker in patients with early and moderately decreased renal function [7–11]. In keeping with this, serum cystatin C showed better performance compared with serum creatinine in studies evaluating patients with diabetes [12–14]. However, in none of these studies, GFR estimations were age-adjusted despite that GFR decreases with increasing age both when evaluated by  $^{51}\text{Cr}$ -EDTA clearance [15] or serum cystatin C [16, 17].

The aim of the current study was to compare serum cystatin C versus serum creatinine (enzymatically determined) as markers for early deterioration of GFR in a large cohort of patients with type 1 and 2 diabetes. In addition, the effect of age on the two GFR markers was evaluated to separate the effects of age from disease.

## Patients and methods

### Controls

Participants in the 'Kristianstad survey' constituted the controls for cystatin C and creatinine [18, 19]. These participants were mainly randomly selected from demographic lists of subjects aged

20–88 years, born on the 20th of any month. Of 359 invited individuals, 249 with an equal sex ratio (124 men and 125 women) and even age distribution (20–88 years), agreed to participate and were evaluated with serum cystatin C and serum creatinine.

Reference values for GFR based on  $^{51}\text{Cr}$ -EDTA were obtained from Granérus and included 503 control subjects aged 20–80 years in whom a close correlation between GFR and age has previously been shown. Before the age of 50, GFR decreases by  $4 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  per decade whereas after the age of 50, the decline is  $10 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  per decade [15].

### Diabetic patients

The patients participated in two prospective studies of type 1 [20] and type 2 [21] diabetes. A total of 41 (27 males and 14 females) of 48 eligible type 1 diabetic patients and 82 (56 males and 26 females) of 90 eligible type 2 diabetic patients were included (Table 1). Measurements for serum creatinine, serum cystatin C, Cr-EDTA clearance, and urinary albumin excretion were performed in all participants. The Ethics committee of Lund University approved the study. Informed consent was obtained from all patients prior to enrolment.

### Laboratory methods

Serum creatinine was determined by an enzymatic method (Kodak Ektachem 700 XR-C system,

**Table 1** Characteristics of patients with type 1 and 2 diabetes

	Type 1	Type 2	P-values
No	41	82	
Age (years)	45 (30–67)	61 (48–72)	<0.0001 <sup>a</sup>
Males/females	27/14	56/26	0.839 <sup>b</sup>
Disease duration (years)	25 (13–42)	9 (5–33)	<0.0001 <sup>a</sup>
$^{51}\text{Cr}$ -EDTA clearance ( $\text{mL min}^{-1} 1.73 \text{ m}^{-2}$ )	102 (26–153)	97 (18–134)	0.279 <sup>a</sup>
S-cystatin C ( $\text{mg L}^{-1}$ )	0.95 (0.79–1.92)	1.03 (0.87–3.69)	0.076 <sup>a</sup>
S-creatinine ( $\mu\text{mol L}^{-1}$ )	86 (69–179)	81 (52–280)	0.566 <sup>a</sup>
Urinary albumin ( $\mu\text{g min}^{-1}$ )	20 (3–3175)	11 (0–2892)	0.918 <sup>a</sup>
No patients with iohexol clearance <60 $\text{mL min}^{-1}$	5	10	1.00 <sup>b</sup>
No patients with iohexol clearance <80 $\text{mL min}^{-1}$	8	15	0.999 <sup>b</sup>

Values expressed as median (range). Statistical calculation of differences between type 1 and 2 diabetic patients was estimated from <sup>a</sup>independent samples *t*-test and <sup>b</sup>chi-square test with Fischer's test.

Eastman Kodak co, Rochester, NY, USA) using the enzymes creatinine amidohydrolase and creatinine amidinohydrolase. Total analytical (intra-assay + inter-assay) imprecision of this method was 2.6% (using a control sample with a concentration of 80  $\mu\text{mol L}^{-1}$ ). Reference range: 55–116  $\mu\text{mol L}^{-1}$  (men), 45–100  $\mu\text{mol L}^{-1}$  (women).

Serum cystatin C was measured by a fully automated particle-enhanced turbidimetric assay on undiluted samples [8]. Reagents were obtained from DAKO (Dako A/S, Glostrup, Denmark) and the determination was performed on a Cobas Mira Plus Instrument (Roche, Basel, Switzerland). As hyperlipidaemia disturbs the measurement, all samples were centrifuged for 10 min at 14 000 *g* and at 4 °C to obtain clear samples for the determination. Total analytical (intra-assay + inter-assay) imprecision of the method was 3.2% (using a control sample with a concentration of 1.28  $\text{mg L}^{-1}$ ). Reference range: 0.6–1.20  $\text{mg L}^{-1}$  (1–50 years), 0.6–1.55  $\text{mg L}^{-1}$  (>50 years) [16].

#### *Glomerular filtration rate*

The GFR was assessed by the  $^{51}\text{Cr}$ -EDTA plasma clearance method. After i.v. injection of 3.7 MBq  $^{51}\text{Cr}$ -EDTA (Cis-bio Int., Gif-Sur-Yvette Cedex, France) four blood samples were drawn at 180–240 min after injection. The plasma was measured for activity in an automatic sample changer (Wallac 1480 Wizard, Wallac, Finland). The plasma clearance was calculated from the quotient between the administered dose and the AUC and was normalized to 1.73  $\text{m}^2$  body surface area [22]. Reference values were obtained from Granérus [15]. The  $^{51}\text{Cr}$ -EDTA plasma clearance method was not changed throughout the study.

#### *Urinary protein analysis*

Urine was collected during two separate nights in connection with the clinical investigation of the patient, and the volume and sampling time were noted. The urine concentration of albumin was determined by radioimmunoassay in both of the samples and the mean value of the two sampling periods were used. U-albumin was expressed as  $\mu\text{g min}^{-1}$  sampling time. Microalbuminuria was defined as urinary excretion of 20–200  $\mu\text{g min}^{-1}$  and macroalbuminuria as >200  $\mu\text{g min}^{-1}$ .

#### *Statistics*

Type 1 and 2 diabetic patients were analysed as one group, as well as separately. All data are presented as median and range unless otherwise stated. Independent samples *t*-test and chi-square test with Fischer's test were used for statistical calculation of differences between type 1 and 2 diabetic patients.

#### *Nonage-adjusted estimations*

Reciprocal values for serum creatinine and serum cystatin C, respectively, were correlated with  $^{51}\text{Cr}$ -EDTA clearance. This method results in linearization of the curvilinear relationship between GFR and serum marker concentration. Correlations were tested using Spearman ( $r_s$ ) and Pearson ( $r$ ), respectively, when appropriate according to distribution. We evaluated the diagnostic accuracy of the individual markers by creating receiver operating characteristic (ROC) curves. Nonparametric ROC curves were generated by plotting the sensitivity versus 1 – specificity, giving the ideal test a sensitivity equal to 1 and a specificity equal to 1 (corresponding to 1 – specificity equal to zero). The AUC describes the diagnostic efficiency of the test. We calculated AUC, 95% confidence interval (CI) and differences between ROC curves [23]. In creating ROC curves we used two cut-off levels for Cr-EDTA clearance, <60 and 80  $\text{mL min}^{-1} 1.73 \text{ m}^{-2}$ , respectively.

#### *Age-adjusted estimations*

Age adjustment was created by using z-scores. This method creates a standardized distribution of normality with a mean of zero. z-Score was calculated from (observed value – reference value)/1 SD [24]. Reference values for serum creatinine and serum cystatin C, respectively, were obtained from median values for every 20 years of healthy controls ( $n = 249$ ). Standard deviation was calculated within this time interval. By using 20-year intervals we maintained the relation between age and renal function and obtained large enough groups to avoid significant variation of the standard deviation. Standard deviation varied from 4.9 to 6.2% of the median values between 20-year intervals for serum creatinine,

and from 4.8 to 6.4% of the median values between the 20-year intervals for serum cystatin C. Age adjusted z-scores for Cr-EDTA clearances were calculated from the previously described reference material [15].

z-Scores for reciprocals of serum creatinine and serum cystatin C were correlated with z-scores for Cr-EDTA clearances. Correlation coefficients ( $r_s$ ) were calculated according to Spearman. We also calculated correlation coefficients ( $r$ ) according to Pearson when appropriate.

Diagnostic accuracy of the individual markers was obtained after age adjustment by creating ROC curves. Decreased renal function was defined at three levels on the basis of z-score for GFR; z-score for GFR  $<-1.64$  SD (95% CI, one-sided interval) for severe renal failure, z-score for GFR  $<-1.28$  SD (90% CI, one-sided interval) for moderate renal failure and z-score for GFR  $<-0.84$  SD (80% CI, one-sided interval) for mild renal failure. Hence, these levels of renal function were chosen from three generally accepted statistical levels.

Statistical analyses were performed with the STATVIEW program version 4.51 (Abacus Concept Inc., Berkeley, CA, USA) and Statistical Package for Social Sciences programs version 10.0 (SPSS Inc., Chicago, IL, USA) on a Macintosh PowerBook G3 computer (Apple Computer Inc., Cupertino, CA, USA).

## Results

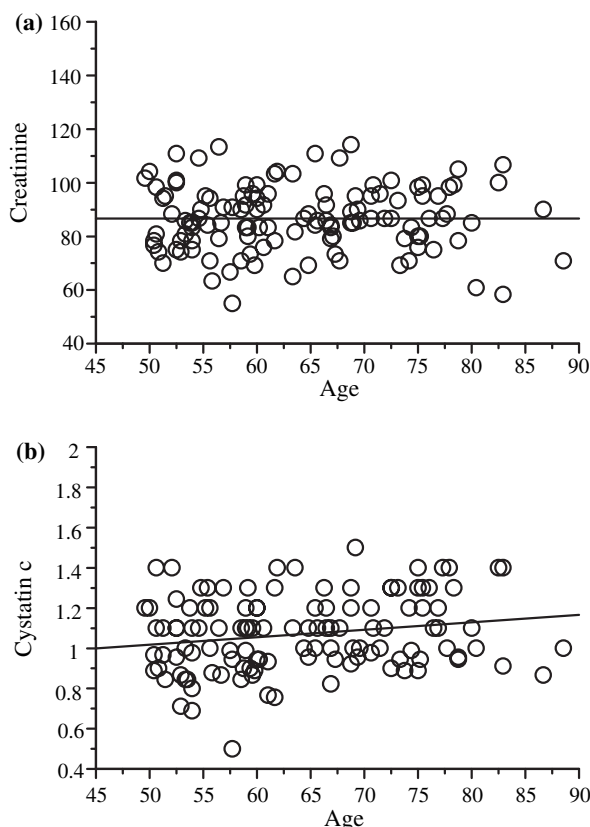
### Diabetic patients

The main characteristics of the type 1 and 2 diabetic patients are summarized in Table 1. Type 1 diabetic patients were significantly younger and demonstrated a longer duration of diabetes compared with type 2 patients. However, there were no significant differences in renal function tests between type 1 and 2 diabetic patients. Sixty-eight patients were normoalbuminuric, 40 patients demonstrated microalbuminuria and 15 patients macroalbuminuria (Table 2). Amongst patients with microalbuminuria only 7–15% displayed decreased renal function defined as either GFR  $<60$  and  $80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$ , respectively or age-adjusted GFR  $<-1.64$ ,  $-1.28$  and  $-0.84$  SD. However, amongst patients with macroalbuminuria 60–80% manifested decreased renal function, defined as above. No significant difference was found between type 1 and 2 diabetes. In patients with macroalbuminuria, 11 displayed increased serum cystatin C and eight increased serum creatinine ( $P = 0.317$ ), according to clinical reference ranges described above. There was a significant but weak relation between rate of albuminuria and decrease in GFR. In type 1 diabetics  $r = 0.412$  ( $P = 0.0082$ ) and in type 2 diabetics  $r = 0.461$  ( $P = 0.0001$ ).

**Table 2** Number of patients with decreased renal function defined by values for Cr-EDTA clearance, cystatin C and creatinine for normoalbuminuria ( $0-19 \mu\text{g min}^{-1}$ ), microalbuminuria ( $20-200 \mu\text{g min}^{-1}$ ) and macroalbuminuria ( $>200 \mu\text{g min}^{-1}$ ). For nonage-adjusted GFR, values for Cr-EDTA clearance  $<60$  and  $80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$ , respectively, were used to define decreased renal function. Age-adjusted GFR was based on z-scores;  $<-1.64$  SD (95% CI) severe,  $<-1.28$  SD (90% CI) moderate and  $<-0.84$  SD (80% CI) for mild renal failure. For serum cystatin C values above  $1.20 \text{ mg L}^{-1}$  ( $1-50$  years) and  $1.55 \text{ mg L}^{-1}$  ( $>50$  years), and for serum creatinine values above  $116 \mu\text{mol L}^{-1}$  (men) and  $100 \mu\text{mol L}^{-1}$  (women), defined decreased renal function. Results show that macroalbuminuria, but not microalbuminuria and normoalbuminuria, were associated with decreased renal function

U-Alb	<i>n</i>	$<60 \text{ mL min}^{-1}$	$<80 \text{ mL min}^{-1}$	$<-1.64 \text{ SD}$	$<-1.28 \text{ SD}$	$<-0.84 \text{ SD}$	Cystatin C	Creatinine
Normoalbuminuria								
Type 1	16	1	2	1	1	2	1	0
Type 2	52	2	5	3	4	4	2	1
	68	3	7	4	5	6	3	1
Microalbuminuria								
Type 1	21	1	2	2	2	3	1	0
Type 2	19	2	2	2	2	3	2	1
	40	3	4	4	4	6	3	1
Macroalbuminuria								
Type 1	4	3	4	4	4	4	4	2
Type 2	11	6	8	8	8	8	7	6
	15	9	12	12	12	12	11	8

Numbers in italics indicate the sum of type 1 and 2 diabetes.



**Fig. 1** Relationship between age and serum creatinine (a) and age and serum cystatin C (b) in persons over the age of 50 years in the control group ( $n = 125$ ).

#### *Age-dependency in the control group*

In the control population, serum creatinine correlated significantly but weakly with age ( $r = 0.158$ ,  $P = 0.0152$ ,  $\beta = 0.11$ ) and increasing with merely  $1.1 \mu\text{mol L}^{-1}$  for every decade in the 249 healthy controls. However, the correlation was absent in the age group  $>50$  years ( $n = 125$ ) ( $\beta = 0.006$ ) (Fig. 1). As for serum creatinine, serum cystatin C increased significantly with age, slightly before ( $\beta = 0.002$ ) and clearly after the age of 50 ( $P = 0.03$ ,  $\beta = 0.004$ ). Serum cystatin C increased by  $0.04 \text{ mg L}^{-1}$  for every decade after 50 years of age (Fig. 1). Hence, there was a clear impact of age on serum creatinine before and for serum cystatin C after the age of 50.

#### *Correlations between endogenous serum markers and GFR*

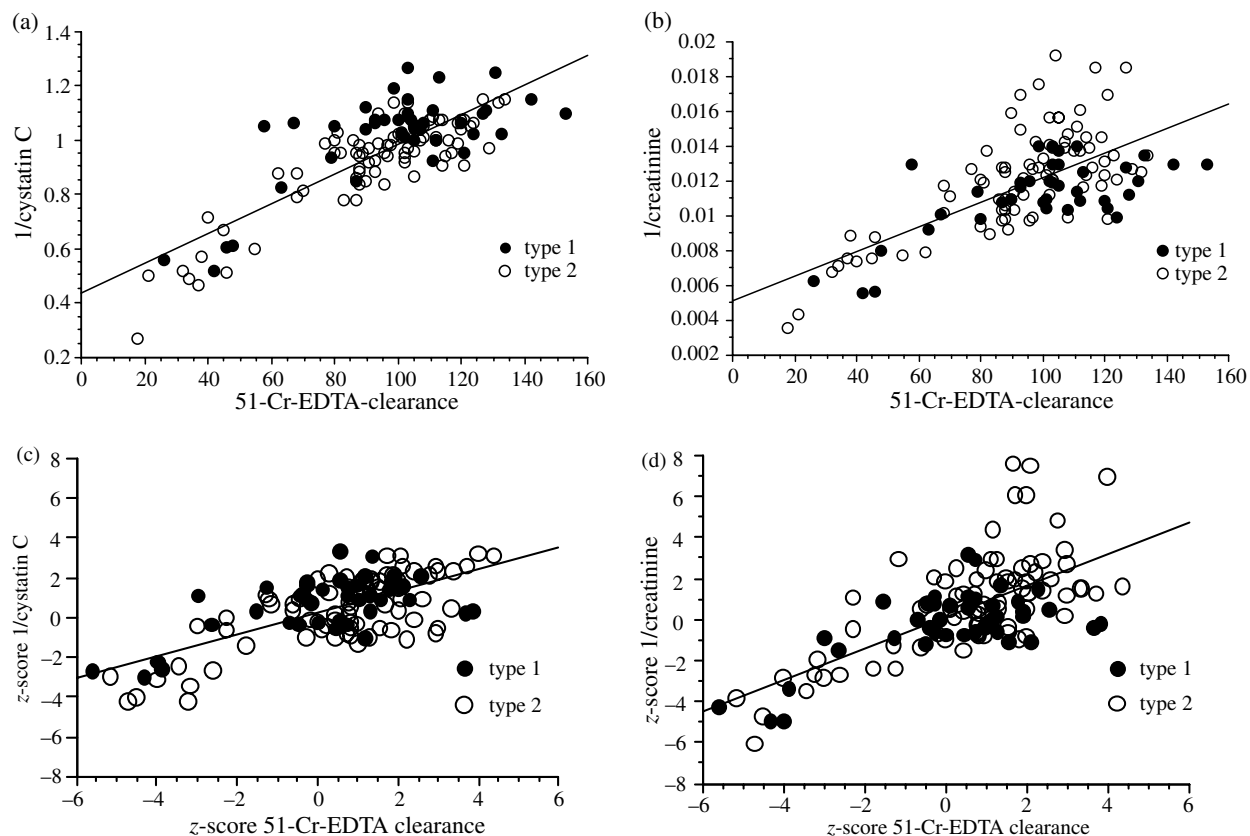
Both in type 1 and 2 diabetic patients ( $n = 123$ ), reciprocals of serum cystatin C correlated

significantly stronger ( $P = 0.0132$ ) with  $^{51}\text{Cr}$ -EDTA clearance ( $r = 0.817$ ,  $P < 0.0001$ ,  $\beta = 0.005$ ) than did reciprocals of serum creatinine with  $^{51}\text{Cr}$ -EDTA clearance ( $r = 0.678$ ,  $P < 0.0001$ ,  $\beta = 0.000071$ ) (Fig. 2 a,b). Hence, using nonage-adjusted values, serum cystatin C correlated significantly better than serum creatinine with GFR as determined by  $^{51}\text{Cr}$ -EDTA clearance.

All estimations were then performed with age adjustment (z-scores) of the GFR markers. In diabetic patients, reciprocals of serum cystatin C and creatinine, respectively, showed equally high correlations ( $r = 0.691$  and  $0.680$ , respectively) (n.s.) to GFR as measured with the age-adjusted  $^{51}\text{Cr}$ -EDTA clearance (Fig. 2c,d). This was shown both in type 1 and 2 diabetic patients. Amongst type 1 diabetic patients, the relationship between age-adjusted serum cystatin C and  $^{51}\text{Cr}$ -EDTA clearance ( $r_s = 0.43$ ,  $P = 0.007$ ) did not statistically differ ( $P = 0.783$ ) from that between age-adjusted serum creatinine and  $^{51}\text{Cr}$ -EDTA clearance ( $r_s = 0.38$ ,  $P = 0.015$ ). In agreement, amongst type 2 diabetic patients the reciprocals of age-adjusted serum cystatin C versus  $^{51}\text{Cr}$ -EDTA clearance ( $r_s = 0.59$ ,  $P < 0.0001$ ) did not differ significantly ( $P = 0.372$ ) from the correlation between reciprocals of age-adjusted serum creatinine versus  $^{51}\text{Cr}$ -EDTA clearance ( $r_s = 0.63$ ,  $P < 0.0001$ ). Hence, when age adjusted, there was no difference between serum cystatin C and serum creatinine in their relationships to  $^{51}\text{Cr}$ -EDTA clearance, both amongst type 1 and 2 diabetic patients.

#### *Diagnostic accuracy*

Diagnostic accuracy based on ROC plots without age adjustment showed that the AUC for serum cystatin C (0.930) was significantly greater than that for serum creatinine (0.683) at a cut-off level  $80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$ , but not at a cut-off level of  $60 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  (Table 3). In keeping with this, also after age adjustment, ROC plots showed significantly greater areas under the curves for serum cystatin C compared with serum creatinine at  $\text{GFR} < -0.84 \text{ SD}$  (0.654 for serum cystatin C and 0.447 for serum creatinine) but not at  $\text{GFR} < -1.28 \text{ SD}$  or  $< -1.64 \text{ SD}$ . Hence, age adjusted or not, serum cystatin C was superior to serum creatinine in the diagnostic accuracy to detect mild nephropathy.



**Fig. 2** Correlation between Cr-EDTA clearance and reciprocal for serum cystatin C (a) and reciprocal for serum creatinine (b), respectively, in type 1 and 2 diabetic patients ( $n = 123$ ) without age adjustment. Linear regression analysis revealed correlation coefficient of 0.817 for (a) and 0.678 (b). Correlation between age-adjusted z-scores for Cr-EDTA clearance versus age-adjusted z-scores for reciprocal of serum cystatin C (c) and age-adjusted z-scores for reciprocal of serum creatinine (d). Linear regression analysis revealed correlation coefficient of 0.691 for (c) and 0.680 for (d) when age adjusted values were considered. Black circles = type 1 diabetes, white circles = type 2 diabetes.

**Table 3** ROC analyses of serum cystatin C and serum creatinine without and with age adjustment in 123 patients with diabetes

	Cut-off levels				
	60 mL min <sup>-1</sup>	80 mL min <sup>-1</sup>	-1.64 SD	-1.28 SD	-0.84 SD
Renal function	1.73 m <sup>-2</sup>	1.73 m <sup>-2</sup>			
Area under the curve					
Serum cystatin C	0.957	0.930*	0.695	0.661	0.654*
Serum creatinine	0.956	0.683*	0.535	0.520	0.447*
95% confidence interval					
Serum cystatin C	0.84–0.97	0.83–0.99	0.55–0.84	0.46–0.78	0.64–0.82
Serum creatinine	0.87–1.0	0.53–0.81	0.24–0.65	0.32–0.72	0.33–0.62

\*Significant difference.

## Discussion

This study found a clear effect of age upon serum cystatin C and serum creatinine concentrations, respectively, in the control material. However, there was a difference between the two GFR markers in

this context. Serum cystatin C was particularly affected after the age of 50 whereas serum creatinine was affected before that age. Hence, albeit the bulk of literature favours that there is a decrease of GFR with age, the effect of age upon renal function has been debated in some studies [25]. Our study

shows that there are differences between different GFR markers in this context. If not adjusted for age, serum cystatin C showed a closer correlation with GFR than serum creatinine. However, when age adjusted, serum creatinine was associated with GFR as closely as serum cystatin C. Sensitivity and specificity for severe impairments in GFR did not differ between age-adjusted serum cystatin C and age-adjusted serum creatinine. The same finding was seen without age adjustment. Hence, serum cystatin C had no major advantage versus age-adjusted serum creatinine in the evaluation of GFR  $<60 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  or  $<-1.28 \text{ SD}$ . However, in the diagnosis of mild diabetic nephropathy (GFR  $< 80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  or age-adjusted GFR  $< -0.84 \text{ SD}$ ), serum cystatin C was more effective than serum creatinine. Our study therefore gives a role for serum cystatin C in the diagnosis of early diabetic nephropathy.

Some previous studies in diabetic patients have shown that serum cystatin C is more sensitive than serum creatinine for detection of GFR in diabetic patients. Harmoinen *et al.* [12] and Mussap *et al.* [13] showed that serum cystatin C was more sensitive than serum creatinine for estimation of GFR in type 2 diabetic patients and Tan *et al.* [14] showed the same in type 1 diabetic patients. Oddo *et al.* [26], on the other hand, demonstrated serum creatinine and serum cystatin C to be equal in diagnostic accuracy in microalbuminuric and proteinuric diabetics. These studies are different from each other in certain aspects. Harmoinen and Mussap, respectively, used an enzymatic creatinine method that is more accurate than the modified Jaffé method [4] but examined only 47 and 52 type 2 diabetic patients, respectively. Oddo *et al.* examined a low number of type 1 and 2 diabetic patients, 15 and 34, respectively, and used a modified Jaffé method for serum creatinine. Tan *et al.* [14] included 29 patients with type 1 diabetes and used an enzymatic creatinine method. In these studies,  $^{51}\text{Cr}$ -EDTA clearance or iothexol clearance were used as reference methods for GFR. However, no age adjustments for GFR were made in any of the studies, although the ranges of age varied up to almost 50 years between examined patients. Instead, decreased renal function was defined at fixed cut-off levels for GFR ( $60$  or  $80 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$ ), irrespective of age. In

our study, we used z-scores for age adjustment and could then show that serum cystatin C and serum creatinine corresponded well in the correlation with GFR and in the establishment of severe nephropathy. Indeed, the advantage of serum cystatin C versus serum creatinine disappeared after age adjustment. Compared with serum creatinine, serum cystatin C was only favourable with regard to the detection of mild nephropathy. In this study, we used a turbidimetric assay for cystatin C. It has been claimed that nephelometric assay for cystatin C performs with a higher accuracy [27]. Thus, if we had used a nephelometric assay, our results may have been strengthened. Furthermore, modified Jaffé methods are common for the assessment of serum creatinine in clinical routine. If a Jaffé method had been employed in our study, the advantage of serum cystatin C versus creatinine would have been more prominent compared with our finding using an enzymatic creatinine method.

Is age adjustment of glomerular function then necessary? This depends on the reason for assessing GFR. Without age adjustment, decreased GFR may denote either a pathological state or the natural ageing of the kidneys. In the detection of early deterioration of GFR because of renal disease, age is certainly an important factor defining the level of normal function. Hence, both in screening for renal diseases and in prospective studies of a GFR marker for renal diseases, age adjustment is necessary. However, if the clinician is interested in evaluating total renal function, e.g. to find adequate doses of drugs in the treatment of elderly patients, age adjustment is not needed.

Besides age adjustments, our study includes the greatest number of type 1 and 2 diabetic patients evaluated by serum cystatin C, so far described. Furthermore, we used the most accurate creatinine method based on enzymatic principles. This may explain why our results showed such a close correlation between both  $1/\text{creatinine}$  and  $^{51}\text{Cr}$ -EDTA clearance as well as between  $1/\text{cystatin C}$  and  $^{51}\text{Cr}$ -EDTA clearance, when age adjustment was performed. However, our finding of a closer correlation for cystatin C than creatinine versus  $^{51}\text{Cr}$ -EDTA clearance, when analyses without age adjustments were conducted, display that age adjustment rather than the creatinine method explains our results.

We found no significant difference in GFR between patients with normoalbuminuria compared with those with microalbuminuria. Only 7–15% of patients with microalbuminuria showed decreased GFR. However, amongst patients with macroalbuminuria, a substantial number demonstrated decreased GFR (60–80%). Neither did serum cystatin C nor creatinine show any changes between normoalbuminuric and microalbuminuric patients. Hence, early changes of diabetic nephropathy, demonstrated by microalbuminuria, are not accompanied by changes in GFR.

Our analyses showed that slightly reduced glomerular function ( $<-0.84$  SD) was detected more accurately with age-adjusted serum cystatin C compared with serum creatinine. This gives a role for serum cystatin C in the early diagnosis of diabetic nephropathy as suggested by Mussap *et al.* [13], but not by Oddo *et al.* [25]. In contrast, age-adjusted serum creatinine was as efficient as age-corrected serum cystatin in the diagnosis of moderate and severe renal insufficiency ( $<-1.28$  SD and  $<-1.64$  SD, respectively). However, our study was aimed to evaluate potential differences between different GFR markers in the early diagnosis or screening of early diabetic nephropathy. Hence, our patient material contained rather few patients with severely decreased renal function. Only 15 patients with  $^{51}\text{Cr-EDTA}$  clearance  $<60 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$  were found in our study. Further studies of patients with severe diabetic nephropathy have to be conducted to confirm our finding. Hence, most of our patients presented with mild nephropathy according to a categorization used by National Kidney Foundation for chronic kidney diseases. This classification with five stages (1–5), uses 90 and  $60 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$ , respectively, as cut-off levels for the two mildest forms of nephropathy.

In conclusion, serum cystatin C is an interesting alternative to serum creatinine as a GFR marker. Our study infers that serum cystatin C is valuable in the detection of early or mild diabetic nephropathy, whereas age-adjusted serum creatinine seems to be as good as serum cystatin C in the diagnosis of severe nephropathy.

### Conflict of interest statement

No conflict of interest was declared.

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