LIVER AND BILIARY DISEASE

Evaluation of serum cystatin C concentration as a marker of renal function in patients with cirrhosis of the liver

A L Gerbes, V Gülberg, M Bilzer, M Vogeser

....

Background and aims: Diagnosis of moderately impaired renal function is of particular importance in patients with cirrhosis of the liver. Whereas patients with a markedly impaired glomerular filtration rate can be diagnosed easily by elevated serum creatinine concentrations, moderately reduced renal function may be missed by this conventional parameter. Recently, cystatin C has been suggested as a sensitive marker of renal function, independent of sex or muscle mass. Therefore, the aim of this study was to investigate the value of serum cystatin C concentration for the detection of moderately impaired renal function.

Methods: Ninety seven inhospital patients with cirrhosis and a 24 hour creatinine clearance of at least 40 ml/min were investigated and divided into group 1 (creatinine clearance ≥70 ml/min; n=55) and group 2 (creatinine clearance 40–69 ml/min; n=42).

Results: Serum cystatin C concentrations (mean (SD): 1.31 (0.51) *v* 1.04 (0.34) mg/l (p=0.008)) and creatinine concentrations (1.03 (0.52) *v* 0.86 (0.22) mg/100 ml (p=0.03)) were higher in group 2 than in group 1; there was no significant difference in urea concentrations. Receiver-operator characteristics (ROC) revealed a differential diagnostic advantage of cystatin C over creatinine and urea. At cut off concentrations of 1.0 mg/l, 0.9 mg/100 ml, and 28 mg/100 ml, respectively, cystatin C, creatinine, and urea exhibited 69%, 45%, and 44% sensitivity (p<0.05). As patients with a small muscle mass or reduced physical activity could be particularly prone to overestimation of their renal function, separate analyses were performed for the subgroups of female and Child-Pugh class C patients, respectively. In both groups, discrimination between patients with moderately impaired and normal renal function was best with cystatin C. In female patients, sensitivity of cystatin C (77.8%) was superior (p<0.05) to that of creatinine (38.9%) and urea (41.2%). In Child-Pugh C patients, the ROC curve was significantly better for cystatin C than for creatinine.

Conclusions: Serum cystatin C determination could be a valuable tool in patients with cirrhosis, particularly with Child-Pugh class C or in female patients, for early diagnosis of moderately impaired renal function.

See end of article for authors' affiliations
....

Correspondence to:
Dr A L Gerbes, Department of Medicine II, Klinikum of the University of Munich-Grosshadern, Marchioninistraβe 15, 81377 Munich, Germany; gerbes@med2.med.uni-muenchen.de

Cirrhosis of the liver is often accompanied by functional renal failure. This is due to haemodynamic alterations, mainly peripheral vasodilatation followed by activation of vasoconstricting hormones and neurohumoral systems such as renin-aldosterone, vasopressin, endothelin, and increased activity of the sympathetic nervous system.[1 2] These alterations induce renal retention of water and sodium, and a decrease in glomerular filtration rate.[1 3] Typically, these impairments in renal function in cirrhosis are of a functional nature which means that they are not accompanied by morphological changes and in the early stages can be reversed by medical intervention.[4–6] The extreme stage of this renal failure however, the hepatorenal syndrome, is rarely reversible, and liver transplantation is the only established therapy.

Patients with functional renal failure of cirrhosis are particularly sensitive to decreases in plasma volume.[7] Therefore, monitoring renal function is pivotal. On diagnosis of deterioration in renal function, appropriate measures such as volume expansion can be taken to avoid further impairment and development of hepatorenal syndrome. Thus indicators of moderately impaired renal function are of great clinical importance. Serum creatinine concentration, the best established simple parameter of glomerular filtration rate, has some disadvantages: its concentration depends on sex and muscle mass and shows marked increases only at severely reduced creatinine clearance values.[8 9] Thus while sufficient for the diagnosis of hepatorenal syndrome,[4] serum creatinine determination can miss less severely impaired renal function. Similar limitations apply for serum urea concentration. Therefore,

rather than determination of serum parameters, measurement of clearance rates of exogenous or endogenous substances has been introduced. Among these, inulin clearance is considered the gold standard but is rarely used because of costs and inconvenience, except in experimental protocols. Creatinine clearance is the most widely used parameter for inhospital patients. It requires 24 hour urine collection and may be inaccurate on an outpatient basis. Thus a simple serum parameter is needed which is sensitive for slight deteriorations in renal function and not influenced by the factors mentioned above.

Recently, the protease inhibitor cystatin C has been suggested as a sensitive marker of glomerular filtration rate and as an early indicator of impaired renal function, possibly superior to serum creatinine.[10 11] Cystatin C is a non-glycosylated low molecular weight protein produced by nucleated cells at a constant rate, freely filtered by the glomeruli, and catabolised in the tubuli. Its renal clearance was found to be very similar to that of exogenous substances such as ^{51}Cr-EDTA.[12] Cystatin C serum concentration appears to be independent of sex or muscle mass and determination is not affected by hyperbilirubinaemia or haemolysis.[13 14] Furthermore, very low intraindividual variation of cystatin C in healthy controls[15] argues against a significant impact of diet on

....

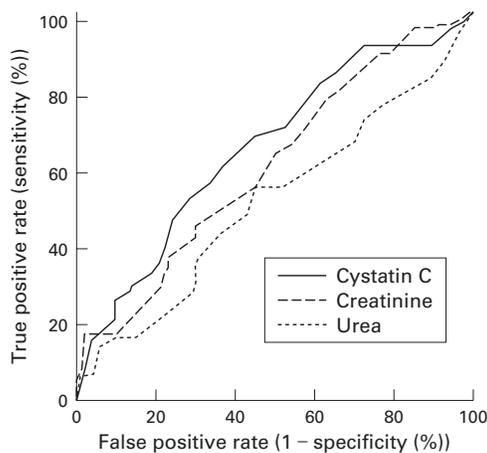**Abbreviations:** ROC, receiver-operator characteristic; AUC, area under the curve.

Figure 1 Receiver-operator characteristic curves for detection of patients with a creatinine clearance less than 70 ml/min. Sensitivity and specificity are displayed at various discrimination levels for serum concentrations of cystatin C, creatinine, and urea. Data were obtained from a total of 97 patients investigated. With increasing differential diagnostic efficiency, the curves approach the left upper corner.
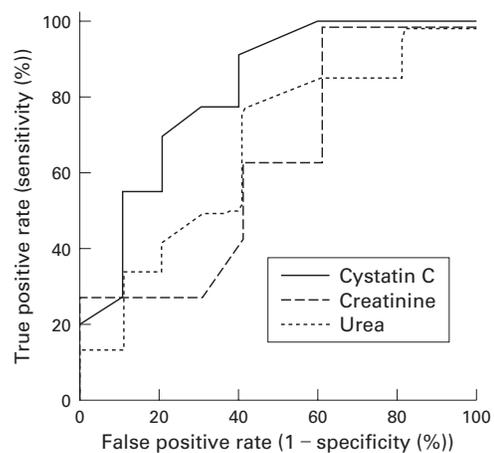

Figure 2 Receiver-operator characteristic curves for serum concentrations of cystatin C, creatinine, and urea for the subgroup of 25 patients with Child-Pugh class C.

serum cystatin C concentrations. The aim of this study was to investigate the value of serum cystatin C in patients with cirrhosis of the liver for detection of moderately impaired renal function.

PATIENTS AND METHODS
Patients
Ninety seven inhospital patients with cirrhosis of the liver and a 24 hour creatinine clearance[16] of at least 40 ml/min were investigated. The 24 hour urine collection on an inpatient basis was carefully controlled. Patients with a creatinine clearance of less than 40 ml/min, the upper limit of the definition of the hepatorenal syndrome,[4] were not included in the study. Mean age was 50 (16) years, and there were 65 males and 32 females. Child-Pugh score was A/B/C in 23/49/25 patients. Aetiology of cirrhosis was alcoholic/post hepatitic/other in 67/19/11 patients.

Laboratory analysis
Serum samples were obtained on the day of urine collection for creatinine clearance and measurement of creatinine, urea, and cystatin C concentrations. Creatinine was analysed by a rate blanked modified Jaffé method.[17] Urea was determined using a kinetic urease method followed by a GLDH-UV test where the decrease in NADH adsorbance is determined photometrically.[9] Both assays (Roche Diagnostic Systems, Mannheim, Germany) were implemented on an Hitachi

multianalyser system. The between run coefficient of variance of these methods was constantly below 3%. Cystatin was determined with the Dade Behring N Latex Cystatin C assay (Dade Behring Diagnostics, Marburg, Germany),[18 19] a particle enhanced nephelometric immunoassay implemented on the Dade Behring Nephelometer II. Intra and interassay coefficients of variation were always below 5% in accordance with earlier reports.[19]

Statistical analysis
Patients were divided into two groups: group 1 had a creatinine clearance of ≥70 ml/min (n=55) and group 2 a creatinine clearance of 40–69 ml/min (n=42). Differences between groups were analysed with the unpaired t test or the Mann-Whitney U test, where appropriate. Data are presented as mean (SD).

Sensitivity, specificity, and diagnostic efficiency were calculated for each value of cystatin C, creatinine, and urea after classification into four categories[20]: true positive (a), true negative (b), false positive (c), and false negative (d). Sensitivity was calculated as a/(a+d)×100, specificity as

Table 1 Sensitivity, specificity, positive and negative predictive value, and efficiency of the serum concentrations of creatinine, urea, and cystatin in discriminating between 42 patients with reduced (group 2) and 55 patients with normal (group 1) creatinine clearance

	Creatinine	Urea	Cystatin C
Cut off value	0.9 mg/100 ml	28 mg/100 ml	1.0 mg/l
Sensitivity (%)	45.2	43.9	69.0* ††
Specificity (%)	70.9	65.5	56.4
Positive predictive value (%)	54.3	48.6	54.7
Negative predictive value (%)	62.9	61.0	70.5
Efficiency (%)	59.8	56.3	61.9

*p<0.05 versus creatinine; ††p<0.01 versus urea; McNemar test.

Table 2 Serum concentrations of creatinine, urea, and cystatin C in female patients and in patients with Child-Pugh class C

	n	Mean (SD)	p Value
Female patients			
Serum creatinine (mg/100 ml)			
Creatinine Cl ≥70 ml/min	14	0.8 (0.2)	0.035
Creatinine Cl 40–69 ml/min	18	0.9 (0.2)	
Serum urea (mg/100 ml)			
Creatinine Cl ≥70 ml/min	14	21.6 (9.4)	0.131
Creatinine Cl 40–69 ml/min	17	31.9 (5.7)	
Serum cystatin C (mg/l)			
Creatinine Cl ≥70 ml/min	14	0.9 (0.3)	0.005
Creatinine Cl 40–69 ml/min	18	1.4 (0.5)	
Child-Pugh class C patients			
Serum creatinine (mg/100 ml)			
Creatinine Cl ≥70 ml/min	11	1.0 (0.2)	0.223
Creatinine Cl 40–69 ml/min	14	1.3 (0.8)	
Serum urea (mg/100 ml)			
Creatinine Cl ≥70 ml/min	11	29.6 (16.2)	0.190
Creatinine Cl 40–69 ml/min	14	42.5 (28.0)	
Serum cystatin C (mg/l)			
Creatinine Cl ≥70 ml/min	11	1.1 (0.3)	0.039
Creatinine Cl 40–69 ml/min	14	1.6 (0.6)	

Creatinine Cl, creatinine clearance.

Table 3 Cystatin C for discrimination between normal and impaired creatinine clearance in the subgroups of 32 female patients and 25 patients with Child-Pugh class C

Cut off value	Creatine 0.9 mg/100 ml		Urea 28 mg/100 ml		Cystatin C 1.0 mg/l	
	Female	*Child-Pugh C*	*Female*	*Child-Pugh C*	*Female*	*Child-Pugh C*
Sensitivity (%)	38.9	60.0	41.2	53.3	77.8*†	86.7
Specificity (%)	92.9	60.0	78.6	60.0	64.3	60.0
Pos predictive value (%)	87.5	69.2	70.0	66.7	73.7	76.5
Neg predicitive value (%)	54.2	50.0	52.4	46.2	69.2	75.0
Efficiency (%)	72.5	60.0	58.1	56.0	71.9	76.0

*p<0.05 versus urea; †p<0.05 versus creatinine; McNemar test.

b/(b+c)×100, positive predictive value as a/(a+c)×100, negative predictive value as b/(b+d)×100, and diagnostic efficiency as (a+b)/(a+b+c+d)×100. The cut off value was then determined at the maximum efficiency derived from analysing all patients. The significance of differences in sensitivity, specificity, or efficiency between the parameters was evaluated by the McNemar test with a two tailed probability. A p value <0.05 was considered statistically significant for all tests applied.

Receiver-operator characteristic (ROC) curves were calculated by standard procedures.[21] The area under the curve (AUC) and 95% confidence interval (CI) were calculated for each plot. The statistical significance of differences between AUC values was determined as proposed recently.[22] Accordingly, a z value was calculated for each comparison and values of z above 1.96 were taken as evidence that ROC areas were different, assuming a two tailed probability.

RESULTS

Creatinine clearance was at least 70 ml/min in 55 patients (group 1) and 40–69 ml/min in 42 patients (group 2). Mean (SD) values for creatinine clearance were 107 (31) ml/min in group 1 and 55 (9) ml/min in group 2 (p<0.0001). Serum concentrations of urea were not significantly different between the two groups: 31.7 (21.4) versus 27.3 (13.3) mg/100 ml. In contrast, serum cystatin C concentrations were higher in group 2: 1.31 (0.51) versus 1.04 (0.34) mg/l (p=0.008). Creatinine concentrations in group 2 exceeded those of group 1: 1.03 (0.52) versus 0.86 (0.22) mg/100 ml (p=0.03).

As illustrated by the ROC curves (fig 1), the diagnostic potential of cystatin C to detect patients with a creatinine clearance of less than 70 ml/min was superior to that of creatinine and urea. At equal specificity, the sensitivity of cystatin C was increased almost throughout the ROC plot. The AUC for cystatin (0.67 (95% CI 0.56–0.78)) was greater than that for creatinine (0.62 (0.51–0.73); z=1.03; NS) and urea (0.53 (0.42–0.66); z=2.68; significant), respectively. At cut off concentrations of 1.0 mg/l, 0.9 mg/100 ml, and 28 mg/100 ml, respectively, cystatin C exhibited significantly higher sensitivity than creatinine and urea (69%, 45%, and 44%; p<0.05) (table 1). Specificity and efficiency were not significantly different between parameters.

Patients with small muscle mass or reduced physical activity could be particularly prone to overestimation of renal function using conventional parameters. We therefore analysed the results in female patients and those with Child-Pugh class C. As shown in table 2, neither serum creatinine nor urea discriminated between Child C patients with a creatinine clearance of more or less than 70 ml/min. In contrast, cystatin C was significantly higher in Child C patients with impaired renal function. ROC curves (fig 2) demonstrated the superiority of cystatin C determination in these patients. AUC for cystatin was greater than that for serum creatinine (0.77 (95% CI 0.58–0.97) v 0.61 (0.37–0.84); z=2.08; significant). Similarly, cystatin C concentrations were more markedly elevated in female patients with a creatinine clearance below 70 ml/min than creatinine and urea (table 2). In this subgroup, AUC for cystatin was also greater than for serum creatinine (0.80 (95% CI 0.64–0.96) v 0.70 (0.60–0.81)) but this was not statistically significant (z=1.82).

For further evaluation of the differential diagnostic value, sensitivity, specificity, positive and negative predictive values, and efficiency were calculated for the subgroups of female and Child-Pugh C patients, respectively (table 3). In Child-Pugh C patients, the sensitivity of cystatin C (86.7%) tended to be higher than that of creatinine (60.0%; NS) and urea (53.3%; NS) at equal specificity of 60%. Overall diagnostic efficiency of cystatin C (76%) tended to be higher than that of creatinine (NS) and urea. In female patients, sensitivity of cystatin C was significantly higher (p<0.05) than that of urea and creatinine.

In Child-Pugh class C patients with a serum cystatin concentration below the cut off value of 1.0 mg/l, no patient had a creatinine clearance below 70 ml/min (fig 3A). In contrast, two patients with a normal serum creatinine concentration (cut off 0.9 ml/dl) exhibited reduced creatinine clearance with values as low as 53 ml/min (fig 3B).
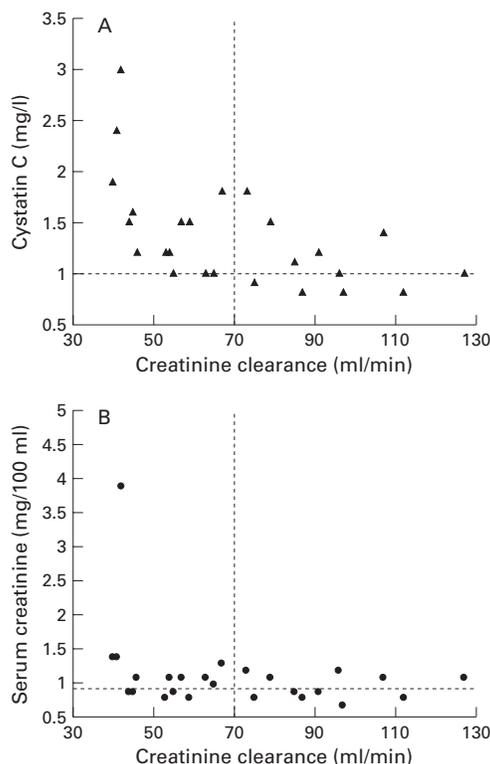

Figure 3 Individual values of cystatin C (A) and serum creatinine (B) in relation to creatinine clearance in 25 patients with Child-Pugh class C. Cut off values are indicated by the broken lines.

DISCUSSION

Cystatin C has recently been introduced as an excellent marker of glomerular filtration rate[11] that is not influenced by several physiological and pathophysiological conditions. While patients with severely impaired renal function exhibit increased serum creatinine concentrations, detection of slightly or moderately decreased glomerular filtration rate by serum parameters is rather difficult.[8] Creatinine clearance, widely used in inhospital patients for estimation of glomerular filtration rate, requires 24 hour urine collection and lacks sufficient reliability in outpatients. Early diagnosis of impaired renal function is particularly important in patients with cirrhosis of the liver. We therefore investigated the diagnostic value of serum cystatin C concentrations in patients with cirrhosis. The following main results were found.

• Serum cystatin C concentrations are significantly increased in patients with cirrhosis and moderately impaired renal function (creatinine clearance 40–69 ml/min) compared with those with creatinine clearance ≥70 ml/min. The difference between these groups is less pronounced for serum creatinine and not significant for serum urea concentrations.

• ROC curves support an advantage of cystatin C over serum concentrations of urea and creatinine.

• In the subgroups of female patients and Child-Pugh C patients, cystatin C was found to be particularly useful for detection of impaired renal function.

Obviously, there was an overlap for concentrations of the renal function parameters between the two groups investigated in our study. This overlap however was smallest for serum cystatin C concentrations. Consequently, as was demonstrated by ROC curves, cystatin C tended to be more sensitive and specific than creatinine throughout almost the whole range of possible cut off values. Urea was even less diagnostically efficient than creatinine. This was further analysed by calculation of sensitivity, specificity, positive and negative predictive values, and efficiency for the most suitable cut off values. Analysis of the 97 patients showed that the sensitivity of cystatin C was superior to that of creatinine and urea. Specificity of cystatin C tended to be lower, but this was not significant. Efficiency however was comparable with the other parameters.

The advantage of cystatin C determination in patients with normal serum creatinine is also supported by the following analysis: 20 of 53 patients with a normal serum creatinine concentration had a decreased creatinine clearance below 70 ml/min. This was the case only in 12 of 45 patients with a normal serum concentration of cystatin C. In addition, 11 of 20 patients with impaired renal function missed by serum creatinine were correctly analysed by cystatin C.

One may argue that renal function in our study was evaluated by 24 hour creatinine clearance rather than by clearance of exogenously administrated substances (for example, inulin clearance). Inulin clearance can reflect glomerular filtration rate more precisely. However, its determination is more cumbersome, requiring a continuous intravenous infusion and urine sampling with a bladder catheter. Thus the majority of clinical investigations of renal function in cirrhosis are based on determination of creatinine clearance. Moreover, recently a correlation between serum cystatin C concentrations and inulin clearance was demonstrated in patients with cirrhosis.[23] Furthermore, creatinine clearance can be as precise as inulin clearance in patients with compensated cirrhosis.[24]

A clear advantage of cystatin C over creatinine or urea determination was found for Child-Pugh C patients. For this subgroup, ROC curves showed a more marked superiority of cystatin C than for the total population. This reflects a trend towards increased diagnostic sensitivity of cystatin C in Child-Pugh C patients at the same specificity levels achieved by the other two parameters. Therefore, in Child-Pugh C patients, cystatin C determinations seem to be of clinical benefit and

should be further investigated. In female patients, the differences in areas under the ROC curves were close to statistical significance. Sensitivity of cystatin C however was significantly higher than that of creatinine and urea at equal specificity. The clear advantage of cystatin C compared with creatinine and urea in these patients may be due to the fact that this parameter is not dependent on muscle mass, activity, or nutritional status.

In summary, in patients with cirrhosis, particularly in patients with Child-Pugh class C, cystatin C determination is a valuable tool for the early diagnosis of moderately impaired renal function.

. . . .

Authors' affiliations
A L Gerbes, V Gülberg, M Bilzer, Department of Medicine II, Klinikum of the University of Munich-Grosshadern, Munich, Germany
M Vogeser, Institute for Clinical Chemistry, Klinikum of the University of Munich-Grosshadern, Munich, Germany

REFERENCES

1 **Møller S**, Henriksen JH. Circulatory abnormalities in cirrhosis with focus on neurohumoral aspects. *Semin Nephrol* 1997;**17**:505–19.
2 **Schrier RW**, Arroyo V, Bernardi M, *et al.* Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988;**8**:1151–7.
3 **Arroyo V**, Gines P, Jimenez W, *et al.* Renal dysfunction in cirrhosis. In: Bircher L, Benhamou JP, McIntyre N, *et al*, eds. *Oxford textbook of clinical hepatology*. New York: Oxford University Press, 1999:733–64.
4 **Arroyo V**, Gines P, Gerbes AL, *et al.* Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. *Hepatology* 1996;**23**:164–76.
5 **Gerbes AL**. Medical treatment of ascites. *J Hepatol* 1993;**17**:S4–9.
6 **Gerbes AL**, Gülberg V, Holl J, *et al.* Renal effects of transjugular intrahepatic portosystemic shunt in cirrhosis: Comparison of patients with ascites, with refractory ascites, or without ascites. *Hepatology* 1998;**28**:683–8.
7 **Bernardi M**, Di Marco C, Trevisani F, *et al.* Renal sodium retention during upright posture in preascitic cirrhosis. *Gastroenterology* 1993;**105**:188–93.
8 **Papadakis MA**, Arieff AI. Unpredictability of clinical evaluation of renal function in cirrhosis. Prospective study. *Am J Med* 1987;**82**:945–52.
9 **Whelton A**, Watson AJ, Rock RC. Nitrogen metabolites and renal function. In: Burtis CA, Ashwood ER, eds. *Tietz textbook of clinical chemistry*. Philadelphia: WB Saunders, 1994:1513–75.
10 **Jung K**, Jung M. Zystatin C: A promising marker of glomerular filtration rate to replace creatinine. *Nephron* 1995;**70**:370–1.
11 **Stickle D**, Cole B, Hock K, *et al.* Correlation of plasma concentrations of cystatin C and creatinine to inulin clearance in a pediatric population. *Clin Chem* 1998;**44**:1334–8.
12 **Tenstad O**, Roald AB, Grubb A, *et al.* Renal handling of radiolabelled human cystatin C in the rat. *Scand J Clin Lab Invest* 1996;**56**:409–14.
13 **Newman DJ**, Thakkar H, Edwards RG, *et al.* Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. *Kidney Int* 1995;**47**:312–8.
14 **Randers E**, Kristensen JH, Erlandsen EJ, *et al.* Serum cystatin C as a marker of the renal function. *Scand J Clin Lab Invest* 1998;**58**:585–92.
15 **Keevil BG**, Kilpatrick ES, Nichols SP, *et al.* Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. *Clin Chem* 1998;**44**:1535–9.
16 **Kampmann J**, Siersbaek-Nilsen K, Kristensen M, *et al.* Rapid evaluation of creatinine clearance. *Acta Med Scand* 1974;**96**:517–20.
17 **Knoll E**, Stamm D. Spezifische Kreatininbestimmung im Serum. *J Clin Chem Clin Biochem* 1980;**8**:582–7.
18 **Finney H**, Newman DJ, Gruber W, *et al.* Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems. *Clin Chem* 1997;**43**:1016–22.
19 **Erlandsen EJ**, Randers E, Kristensen JH. Evaluation of the Dade Behring N Latex Cystatin C assay on the Dade Behring Nephelometer system. *Scand J Clin Lab Invest* 1999;**59**:1–8.
20 **Galen RS**, Gambino SR. *Beyond normality: The predictive value and efficiency of medical diagnosis*. New York: John Wiley, 1975.
21 **Hanley JA**, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;**143**:29–36.
22 **Hanley JA**, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983;**148**:839–43.

23 **Woitas RP**, Stoffel-Wagner B, Flommersfeld S, *et al*. Correlation of serum concentrations of cystatin C and creatinine to inulin clearance in liver cirrhosis. *Clin Chem* 2000;**46**:712–15.

24 **Orlando R**, Floreani M, Padrini R, *et al*. Evaluation of measured and calculated creatinine clearances as glomerular filtration markers in different stages of liver cirrhosis. *Clin Nephrol* 1999;**51**:341–7.

Want full text but don't have a subscription?

Pay per view

For just $8 you can purchase the full text of individual articles using our secure online ordering service. You will have access to the full text of the relevant article for 48 hours during which time you may download and print the pdf file for personal use.

www.gutjnl.com