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EFFECTS OF GLUCOCORTICOID IMMUNOSUPPRESSION ON SERUM CYSTATIN C LEVELS

UTICAJ GLUKOKORTIKOIDNIH IMUNOSUPRESIVA NA NIVO CISTATINA C U SERUMU

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Summary: The aim of the present study is to describe the influence of glucocorticoid immunosuppression on serum cystatin C concentration in renal transplant patients. To evaluate the influence of immunosuppressive regimens, especially glucocorticoids, on serum cystatin C level, 38 clinically stable patients on immunosuppression therapy with low-dose glucocorticoids were compared to 30 clinically stable patients receiving cyclosporin A alone, and 18 clinically stable patients receiving cyclosporin A together with azathioprine. Clinical stability was defined as the absence of acute rejection, febrile infection, and cyclosporin A toxicity, as well as stability of creatinine clearance as estimated by the formula of Cockroft and Gault. All groups were compared for estimated creatinine clearance (CrCl) values and had comparable gender, age and time since transplantation. The group receiving short-course, high-dose methylprednisolone was analyzed at four time points: a) before methylprednisolone commencement (median, 15 days); b) the day methylprednisolone was introduced (before medication); c) after 3 days of methylprednisolone therapy; and d) on a follow-up 9–10 days after the last dose. Intravenous administration of high-dose methylprednisolone led to significant differences in cystatin C levels at different time points (before administration, after three doses, and 8 days after discontinuation). Glucocorticoid medication in adult renal transplant patients is associated in a dose-dependent manner with increased cystatin C, leading to systematic underestimation of GFR. Moreover, our data illustrate the need for specific reference intervals in patients on glucocorticoid therapy. In clinical routine settings, as well as in future clinical studies, it is important to take glucocorticoid medication into account when interpreting serum cystatin C concentrations in renal transplant patients presumably, as well as in other patient groups.

Keywords: cystatin C, renal transplantation, methylprednisolone

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Kratak sadržaj: U radu je opisan uticaj glukokortikoidne imunosupresije na koncentraciju cistatina C u serumu pacijenata posle transplantacije bubrega. Kako bi se odredio uticaj imunosupresivne terapije, naročito glukokortikoida, na nivo cistatina C u serumu, upoređeno je 38 klinički stabilnih pacijenata koji su primali niske doze glukokortikoida sa 30 klinički stabilnih pacijenata koji su primali samo ciklosporin A, i 18 klinički stabilnih pacijenata koji su primali ciklosporin A zajedno sa azatioprinom. Klinička stabilnost je definisana kao odsustvo akutne reakcije, febrilne infekcije i ciklosporinske nefrotoksičnosti uz stabilan klirens kreatinina utvrđen pomoću formule Kokrofta i Golta. Grupa pacijenata koji su primali u kratkom periodu visoke doze metilprednizolona analizirana je 15 dana ranije, na dan aplikacije, trećeg i 9–10 dana po završetku terapije. Rezultati su potvrdili da u prva tri dana od primene (500 mg/dan) postoji značajno povećanje koncentracije cistatina C, koja se normalizovala po završetku terapije. Rezultati dobijeni primenom niskih doza glukokortikosteroidne terapije pokazuju značajno povećanje koncentracije cistatina C u odnosu na kontrolnu grupu. Ova preliminarna ispitivanja ukazuju na potrebu uvođenja specifičnog referentnog intervala za pacijente na glukokortikoidnoj terapiji.

Ključne reči: cistatin C, transplantacija bubrega, metilprednizolon

Introduction

Cystatin C (Cys C) is a nonglycosylated basic protein (13.36 kDa) that can be found in a variety of biologic fluids (1). It has been documented that cystatin C possesses many characteristics of an ideal GFR marker (e.g. endogenously produced at a constant rate, freely filtered in the glomerulus, neither

reabsorbed nor secreted in the renal tubule, not extrarenally eliminated) and has been reported to be at least as accurate as the commonly used serum creatinine to detect impaired renal function in various patient groups, including renal transplant patients (2–5). The comparison between creatinine, cystatin C and β-trace protein (BTP), another small, freely filtered protein, clearly shows higher sensitivity of CysC and βTP in the early stages of disease (6). After renal transplantation, patients are at risk of acute damage to the transplanted kidney because of rejection or toxicity from immunosuppressant therapy. Therefore, early detection of renal damage may lead to more effective intervention. In a preliminary study, LeBricon et al. (7) were the first to suggest that CysC was more sensitive than SCr for detecting decreases in GFR and delayed graft function in renal transplant patients. As in most studies, plasma CysC measurements correlated well with SCr and CrCl.

In recent studies, cystatin C has been introduced as a new potential marker that provides more accurate estimation of GFR (6). Cystatin C is a 13 kDa endogenous cysteine proteinase inhibitor produced by all nucleated cells at a constant rate and broken down completely in the renal tubuli (8). Its concentrations are independent of age and body weight, and there is no need for urine collection for clearance estimations. Furthermore, cystatin C serum concentrations are not influenced by malignancy or inflammation. In contrast, the often-used serum creatinine concentration is supposed to be influenced by dietary intake, renal tubular metabolism, age, and muscle mass.

Serum cystatin C is a more appropriate and effective biomarker for the overall estimation of GFR than serum creatinine values (9). Corticosteroids are among the most commonly prescribed drugs in nephrology. Therefore, a possible interaction of corticosteroid therapy with serum cystatin C concentration or cystatin C measurement is highly relevant for its potential as a marker of GFR.

Despite this, Bokenkamp et al. (10) reported that cystatin C levels are higher in children with renal transplants than in children with other renal pathologies, but that both groups have comparable GFRs, suggesting an underestimation of GFRs by cystatin C in renal transplant patients. This finding indicated immunosuppression as a major influencing factor because all patients had received prednisolone and cyclosporine A medication. However, neither prednisolone nor cyclosporine A were believed to induce change in cystatin C concentrations, because no dose-dependent increase of cystatin C was found. In contrast, an in vitro study by Bjarnadottir et al. (11) described a dose-dependent increase of cystatin C production in HeLa cells exposed to dexamethasone.

In a preliminary study, LeBricon et al. (14) first suggested that CysC level was more sensitive than serum creatinine (SCr) concentration for detecting decreases in GFR and delayed graft function in renal transplant patients. As in most studies, plasma CysC measurements correlated well with SCr and creatinine clearance (CrCl).

After renal transplantation, plasma (or serum) creatinine is the most common marker for the assessment of allograft function. In a steady-state muscular mass balance, the plasma creatinine concentration is assumed to reflect glomerular filtration rate (GFR) (15). However, plasma creatinine is far from being an ideal marker of GFR, despite its convenience and low cost (16). Plasma creatinine suffers a high degree of interindividual variability related to sex, age, body composition, and dietary factors (9, 17). With altered renal function, the plasma creatinine concentration increases only when GFR is reduced by > 50%.

Therefore, the aim of the present preliminary study is to elucidate the effects of glucocorticoid immunosuppressive therapy on cystatin C and β_2-microglobulin concentrations in the serum of renal transplant patients, and to indicate them as possible markers of GFR.

Material and Methods

Reference intervals obtained from a cohort of 100 healthy blood donors were used. They were free of cardiac, liver or renal diseases or hypertension and had normal urine and normal serum urea, creatinine, CysC and β_2-microglobulin concentration.

Clinical stability was defined as the absence of acute rejection, febrile infection, and cyclosporin toxicity. Sixty-four patients with end-stage renal disease undergoing renal transplantation were included in this study. Primary diagnoses of the investigated patients were: chronic interstitial nephropathy (n = 8), diabetic glomerulopathy (n = 12), polycystic kidney disease (n = 3), nephrosclerosis (n = 3), focal segmental glomerulosclerosis (n = 20), IgA nephropathy (n = 8), membranous glomerulonephritis (n = 11), mesangiocapillary glomerulonephritis (n = 1), and unknown (n = 4). Immunosuppressive regimen included steroids (methylprednisolone in an initial dose of 500 mg, followed by 1 mg × kg^{-1} × day^{-1}, progressively tapered) and cyclosporine A (initial dose of 8 mg × kg^{-1} × day^{-1}, and then adjusted according to blood concentrations) or FK506 in cases of cyclosporine intolerance (in the dose of 0.1 mg × kg^{-1} × day^{-1}).

Serum cystatin C and β_2-microglobulin were measured by a particle-enhanced turbidimetric immunoassay-PETIA, Dako (18, 19), on the Cobas Mira (Roche) (4, 20). Serum creatinine was measured by a modified kinetic Jaffe method (Dimension RXL; Dade-Behring (21). Creatinine clearance was estimated by the Cockroft and Gault formula (22).

For the calculation of estimated GFR several formulas have been derived from different studies based on cystatin C: the formula published by Hoek in 2003 (23) and the Larsson formula published in 2004 (24).

Data are presented as mean ± SD or as median and interquartile range (IQR) where appropriate (the difference between groups was determined by the Student's t-test). The correlation was analyzed by the Pearson linear regression test. Values of $p<0.05$ were taken as statistically significant.

Results

The mean serum cystatin C, β_2-microglobulin and creatinine concentrations were: creatinine 82.0+/– 24 μmol/L (53.0–106.0), cystatin C 0.93 +/–0.45 mg/L (0.48–1.38), β_2-microglobulin 1.70+/–0.98 mg/L (0.66–2.74).

Serum cystatin C and creatinine concentration were measured in all healthy control patients. Obtained data were analyzed using linear regression. Significant correlation between serum cystatin C and creatinine concentration was observed ($r=0.654$; $p<0.001$) (*Figure 1*).

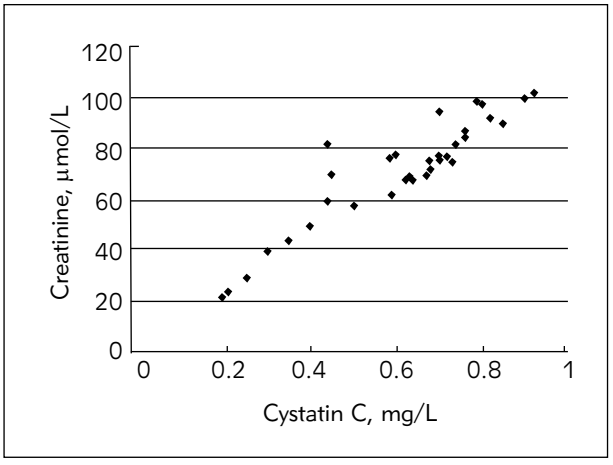

Figure 1 Correlation between serum cystatin C (mg/L) and serum creatinine (μmol/L) in healthy adults.

The patients were checked for routine follow-up (three groups), which included the assessment of clinical data and laboratory results. To evaluate the effects of immunosuppressive regimens, especially glucocorticoids, on serum cystatin C levels, 64 clinically stable patients receiving only cyclosporine A and 42 clinically stable patients receiving immunosuppressant therapy with low-dose glucocorticoids were compared to 16 patients receiving cyclosporin A together with high-dose methylprednisolone (*Table I*).

Since it was noted that patients receiving long-term, low-dose glucocorticoid therapy demonstrated higher cystatin C concentrations than controls, cystatin C kinetics after glucocorticoid administration and withdrawal were investigated.

Intravenous administration of high-dose methylprednisolone led to significant differences in cystatin C values at different time points (before administration, after three doses, and 8 days after discontinuation; $P<0.001$). The group receiving short-course, high-dose methylprednisolone was controlled at four time points available: (a) the visit before methylprednisolone commencement (median, 17 days; range, 2–67 days); (b) the day methylprednisolone was introduced (before medication); (c) after 3 days of methylprednisolone therapy; and (d) on a follow-up visit (median, 8 days after last dose; range, 6–11 days). After a three-day dose of 500 mg, cystatin C concentrations increased from 2.15 mg/L (IQR, 1.60–2.70) to 2.55 mg/L (IQR, 2.35–3.25; $p<0.05$). Eight days after discontinuation, cystatin C concentrations significantly decreased to 2.00 mg/L (1.73–2.15; $p<0.05$).

At these time points, neither the CrCl estimate (54 ± 13 mL × min^{-1} × 1.73 m^{-2}, 51 ± 12 mL × min^{-1} × 1.73 m^{-2}, and 56 ± 14 mL × min^{-2} × 1.73 m^{-2}; $p = 0.08$) nor the serum creatinine concentrations (175 μmol/L,156–202; 178 μmol/L,161–203; and 158 μmol/L, 144–199; $p = 0.17$) underwent significant changes.

Throughout the study period, 16 rejection episodes in 16 different patients took place and were treated. All analytes showed similar behaviour in the days prior to rejection treatment. CysC serum levels paralleled the time course of creatinine in six out of eight patients who did not require dialysis. In one case, the CysC level increased the day before creatinine and, in one further case, the CysC level

Table I Clinical characteristics of renal transplant patients.

Characteristics	Cyclosporine N=64	Low-dose glucocorticoid N=42	High-dose glucocorticoid N=16
Time after transplantation, years	4.7+/–2.5	6.2 +/–2.7	5.8+/-3.6
Creatinine clearance, mL/min	51.5+/–9.5	46+/–15*	44.0+/–10.0*
Cystatin C, mg/mL	1.65* 1.21–1.98	2.95* 1.90–2.90	2.25* 2.34–3.50

* Significance compared with control group

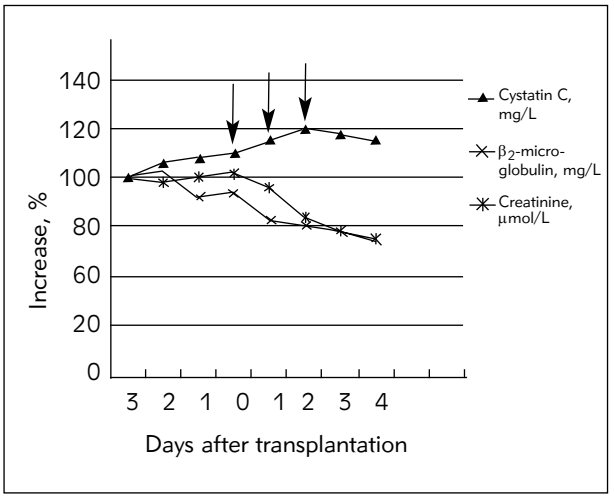

Figure 2 Changes in cystatin C, creatinine and β_2-microglobulin levels during rejection treatment. Arrows indicate rejection treatment with high-dose methylprednisolone (500 mg each).

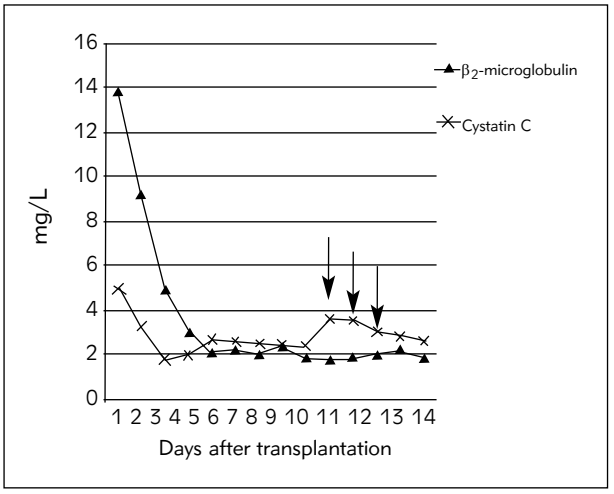

Figure 3 Time course of serum cystatin C and β_2-microglobulin levels in patients during study period. Arrows indicate rejection treatment with high-dose methylprednisolone.

remained stable. The time course of β2MG showed similar results. The 16 antirejection therapies were performed via daily administration of 500 mg of methylprednisolone intravenously for 3 days. On average, rejection treatment was initiated 8 days after transplantation. Rejection therapy resulted in a significant decrease (14.6±5.4%) of the creatinine level in all patients who were not dialysed. β_2MG levels were not significantly altered by the treatment and therefore decreased slightly (–8.4±6.89%; 5.0±1.2 vs. 4.3±0.81 mg/L, NS). In contrast, in seven out of eight patients who did not require dialysis an increase in CysC levels (22.8±7.9%; $p<0.05$) was noted (*Figure 2*).

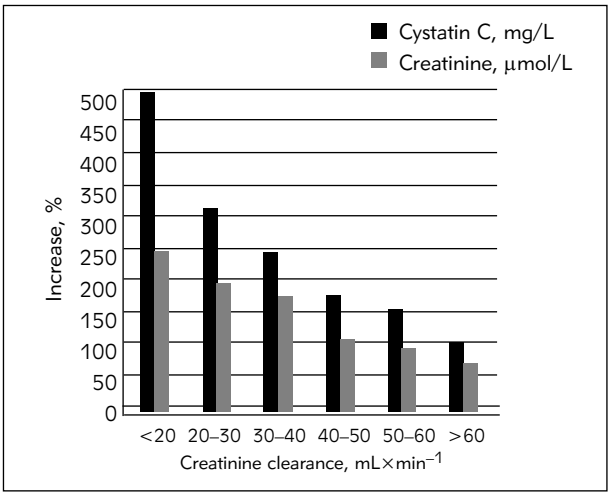

Figure 4 Cystatin C and creatinine concentration in a transplant patient with different clearance value range.

Although low flux dialysis reduced CysC concentrations, the five patients on dialysis also showed an increase in CysC levels (8.0±4.2%). Following the steroid bolus, the highest CysC levels occurred on the second day of treatment. To point out the time course of the analytes more clearly a representative sample of these patients is shown in *Figure 3*.

All investigated patients with renal rejection were classified into groups with different clearance results: <20, 20–30, 30–40, 40–50, 50–60, and >60 mL × min^{-1} (1.73 m^2)$^{-1}$. The relative increases in analytes (cystatin C and creatinine) above the upper reference value determined for each subgroup are shown in *Figure 4*.

Discussion

This study demonstrates that renal transplant patients receiving glucocorticoid medication have higher cystatin C levels than the group treated with glucocorticoid-free immunosuppressives. Since patients receiving 500 mg of methylprednisolone had significantly higher cystatin C values than patients receiving 10 mg of prednisolone, a dose-dependent influence of the administered glucocorticoid dose is suggested.

Thus, glucocorticoid medication leads to systematic underestimation of GFR in renal transplant patients. The hyperbolic relationship deriving from linear regression lines allows the estimation of cystatin C increase at specific levels of CrCl.

Similar to our findings, Bjarnadottir et al. (11) observed that dexamethasone caused a dose-dependent increase in the cystatin C secretion of cultivated HeLa cells. Furthermore, when an expression system was transfected in HeLa cells by chimeric plasmid constructs of the cystatin C promoter coupled

to the structural gene coding for the human growth hormone, a statistically significant increase of human growth hormone secretion after dexamethasone administration could be detected. These findings suggest that the glucocorticoid-induced increase in cystatin C production reflects a promoter-mediated increase in transcription of the cystatin C gene. Despite these in vitro results, cystatin C serum concentration in vivo is thought to be mainly determined by GFR, although some exceptions have been reported. A similar effect of glucocorticoid medication has been reported in asthmatics by Cimerman et al. (25).

In comparison to our observations, in renal transplant patients receiving a 3-day course of 500 mg of methylprednisolone per day, they found a highly significant increase in cystatin C after 1 week of 40 mg of methylprednisolone daily. The present study demonstrates that the increase in cystatin C concentration is a transitory phenomenon, because after a median of 8 days after the cessation of methylprednisolone, a decrease was observed before high-dose glucocorticoid administration. Bokenkamp et al. (10) reported that serum cystatin C in pediatric renal transplant patients is higher than in nonrenal transplant children with comparable GFR. It was not stated, however, how many participants in the control group had received glucocorticoid-free medication. Furthermore, the control group was compared only for GFR, age, and gender, but not for kidney transplantation. Therefore, it was not possible to determine whether glucocorticoid medication or renal transplantation induced the difference in cystatin C concentrations. In contrast to our observations, a dose-dependent influence of glucocorticoid medication on cystatin C could not be demonstrated, probably because the differences in individual prednisolone doses were too small to reflect in the cystatin C concentrations. Risch et al. (26) investigated the role of CysC in 30 renal transplant patients. CysC was superior to SCr and β_2-microglobulin ($p = 0.025$), but it had a positive predictive value for detecting a GFR <60 mL/min determined by [^{125}I]iothalamate clearance similar to that of a 24-h CrCl ($p = 0.76$). With an upper reference value of 1.64 mg/L, the sensitivity and specificity of CysC were 70% and 89%, respectively, producing a positive predictive value of 93%. SCr (upper reference limit, 125 μmol/L) had a positive predictive value of 76%, whereas CrCl had a positive predictive value of 94%. In a prospective study of 110 consecutive adult patients, no statistical differences were found between CysC and SCr levels for detecting impaired GFR determined by CrCl (27). However, the authors pointed out the flaw of not having any gold standard GFR determinant. Finally, among 24 pediatric renal transplant patients, CysC levels were not able to predict acute rejection any sooner than SCr in the 9 patients who suffered (28).

In general, glucocorticoid medication in adult renal transplant patients is associated in a dose-dependent manner with increased cystatin C, leading to systematic underestimation of GFR. This does not preclude the use of cystatin C in detecting impaired renal function in renal transplant patients with glucocorticoids, because our, and other studies (17, 29) showed cystatin C to be significantly more accurate in detecting impaired renal function in this patient group. Moreover, our data illustrate the need for specific reference intervals in patients on glucocorticoid therapy. Depending on steroid dose and CrCl impairment, the cystatin C increase can be estimated to be 0.20–1.85 mg/L in patients receiving low-dose glucocorticoids and even higher in patients receiving high-dose glucocorticoids. However, a more detailed study evaluating dose effects is needed. In conclusion, in clinical routine settings, as well as in future clinical studies, it is important to take glucocorticoid medication into account when interpreting serum cystatin C concentrations in renal transplant patients.

References

1. Grubb AO. Cystatin C – properties and use as diagnostic marker. Adv Clin Chem 2000; 35: 63–99.
2. Finney H, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. Ann Clin Biochem 2000; 37: 49–59.
3. Swan SK. The search continues – an ideal marker of GFR [Editorial]. Clin Chem 1997; 43: 913–14.
4. Tanaka A, Suemaru K, Araki H. A new approach for evaluating renal function and its practical application. J Pharmacol Sci 2007; 105: 1–5.
5. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C – a new marker of glomerular filtration rate in children independent of age and height. Pediatrics 1998; 101: 875–81.
6. Filler G, Priem F, Lepage N, et al. β-Trace protein, cystatin C, β_2-microglobulin and creatinine compared for detecting impaired glomerular filtration rates in children. Clin Chem 2002; 48: 729–36.
7. Le Bricon T, Thervet E, Froissart M, Benlakehal M, Bousquet B, Legendre C, Erlich D. Plasma cystatin C is superior to 24-h creatinine clearance and plasma creatinine for estimation of glomerular filtration rate 3 months after kidney transplantation [Letter]. Clin Chem 2000; 46: 1206–7.
8. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T, Grubb AO, Price CP. Serum cystatin C: a replacement for creatinine as a biochemical marker of GFR. Kidney Int 1994; 46: S20–S21.
9. Hillege HL, Verhave JC, Bakker SJL, et al. Cystatin C is

superior to serum creatine in prediction of mortality in the general population; data obtained from the PREVEND study. ASN congress 2003; Abstract SA-FC 150.

10. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C serum concentrations underestimate glomerular filtration rate in renal transplant recipients. Clin Chem 1999; 45: 1866–8.
11. Bjarnadottir M, Grubb A, Olafsson I. Promoter-mediated, dexamethasone-induced increase in cystatin C production by HeLa cells. Scand J Clin Lab Invest 1995; 55: 617–23.
12. Kalansoorija A, Holbrook I, Jennings P, et. al. Serum cystatin C, enzymuria, tubular proteinuria and early renal insult in type 2 diabetes. Br J Biomed Sci 2007; 64: 121–3.
13. Randers E, Erlandsen EJ, Pedersen OL, Hasling C, Danielsen H. Serum cystatin C as an endogenous parameter of the renal function in patients with normal to moderately impaired kidney function. Clin Nephrol 2000; 54: 203–9.
14. Le Bricon T, Tervet E, at al. Changes of Plasma Cystatin C after Renal Transplantation and Acute Rejection in Adults. Clin Chem 1999; 44: 2243–9.
15. Herget-Rosenthal S, Trabold S, Huesing J, Heemann U, Philipp T, Kribben A. Cystatin C – an accurate marker of glomerular filtration rate after renal transplantation? Transpl Int 2000; 13, 285–9.
16. Gowans E, Fraser CG. Biological variation of serum and urine creatinine and creatinine clearance: ramifications for interpretation of results and patient care. Ann Clin Biochem 1988; 25: 259–63.
17. Roos JF, Doust J, Tett SE, et al. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children. A meta analysis. Clin Biochem 2007; 40: 383–91.
18. Newman D, Thakkar H, Edwards R, Wilkie M, White T, Grubb A, Price C. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. Kidney Int 1995; 47: 312–8.
19. Kyse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 1994; 40: 1921–6.
20. Helin I, Axenram M, Grubb A. Serum cystatin C as a determinant of glomerular filtration rate in children.1998; Clin Nephrol 49, 221–5.
21. Knapp ML, Mayne PD. Development of an automated kinetic Jaffé method designed to minimise bilirubin interference in plasma creatinine assays. Clin Chim Acta 1987; 168: 239–46.
22. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31–4.
23. Hoek FJ, Kemperman FAW, Ktediet RT. A comparison between Cystatin C, plasma creatinine and the Cocroft and Gault formula for the estimation of glomerular filtration rate. Nephrol Dial Transplant 2003; 18: 2024–31.
24. Larson A, Malm J, Grubb A, et al. Calculation of glomerular filtration rate expressed im mL/min prom plasma Cystatin C values in mg/L. Scand J Clin Lab Invest 2004; 64: 25–30.
25. Cimerman N, Brguljan PM, Krasovec M, Suskovic S, Kos J. Serum cystatin C, a potent inhibitor of cysteine proteinases, is increased in asthmatic patients. Clin Chim Acta 2000; 300: 83–95.
26. Risch L, Blumberg A, Huber A. Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C. Nephrol Dial Transplant 1999; 14: 1991–6.
27. Bokenkamp A, Ozden N, Dieterich C, Schumann G, Ehrich JHH, Brodehl J. Cystatin C and creatinine after successful kidney transplantation in children. Clin Nephrol 1999; 52: 371–6.
28. Fliser D, Ritz E. Serum cystatin C concentration as a marker of renal dysfunction in the elderly. Am J Kidney Dis 2001; 37: 79–83.

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