Serum cystatin C is an easy to obtain biomarker for the onset of renal impairment in heart transplant recipients

Daniela Kniepeiss, MD,^a Doris Wagner, MD,^a Gerhard Wirsberger, MD,^b Regina E. Roller, MD,^b Andrä Wasler, MD,^a Florian Iberer, MD,^a and Karl-Heinz Tscheliessnigg, MD^a

Objective: With the increasing longevity of heart transplant recipients, the long-term effects of cyclosporine on renal function have become more evident. Highly sensitive, early, and effective monitoring of posttransplant renal function is still being researched. This study aimed to evaluate the prognostic value of cystatin C for patients after heart transplantation.

Methods: Seventy-three long-term recipients of a heart transplant more than 5 years before the study start were included in the analysis with a follow-up of 4 years. Serum creatinine, renal glomerular filtration rate calculated by the Modification of Diet in Renal Disease formula, and serum cystatin C levels were collected, and risk factors for renal dysfunction were assessed. Statistical analysis was performed for all patients.

Results: Univariate analysis showed a prognostic impact of antihypertensive medication and onset of diabetes ($P < .001$) on renal failure after transplantation. Multivariate analysis yielded cystatin C measured at the study start as a superior prognostic parameter for all time points (area under the receiver operating characteristic 12 months: 0.963; 24 months: 0.910; 48 months: 0.949) compared with the conventionally used creatinine levels.

Conclusions: Our results showed an enormous potential of serum cystatin C as an early prognostic and easy to obtain biomarker for renal dysfunction after heart transplantation. (J Thorac Cardiovasc Surg 2010;140:688-93)

Renal dysfunction is one of the most common and threatening complications in patients after heart transplantation (HTx). Renal failure after the transplantation of a nonrenal organ complicates medical management and leads to increased morbidity and mortality.^{1,2} The risk of chronic failure and the need for long-term renal replacement therapy have increased and will increase further because of ameliorated longevity in the overall population of transplant recipients. A direct proportion of an increasing rate of chronic renal failure with the longevity of HTx recipients has already been shown.³

As reported previously, approximately 7% of patients become dialysis dependent within 5 to 10 years after HTx. Furthermore, the occurrence of chronic renal failure among patients with a nonrenal transplant has been associated with a more than 4 times higher mortality.⁴ Kidney disease in nonrenal transplant recipients may also trigger the occurrence of concomitant diseases, such as osteoporosis, anemia, and hypertension.

A crucial point for studies on renal function after HTx is the careful measurement of renal function itself. Serum

creatinine (sCr) is widely used as marker for renal function, but it depends on various nonrenal factors such as muscle mass, physical activity, and protein intake.⁵ Furthermore, major changes occur late in the course of progressive renal impairment. Usual markers of kidney function, such as sCr, creatinine clearance, and calculated creatinine clearance using the Cockcroft–Gault equation, have been shown to be inaccurate in predicting a glomerular filtration rate (GFR) less than 80 mL/min/1.73 m².⁶ The use of cystatin C as a marker of GFR was first proposed in 1985.⁷ Cystatin C is produced by nucleated cells at a constant rate.⁸ It is cleared from the blood by glomerular filtration and is then reabsorbed and catabolized in proximal tubular cells without reentering the circulation.⁹ Therefore, cystatin C has been suggested to be an excellent marker of GFR,¹⁰ unrelated to weight and gender and unaltered by inflammatory conditions.¹¹

The present study evaluated the prognostic impact of cystatin C in a population of recipients 5 to 10 years after HTx. To find an easy to obtain parameter for the prediction of late renal failure means to minimize patients' risk for dialysis-dependent renal failure and to optimize patients' graft survival through the reduction of the risk for the onset of immunosuppressive-associated renal hypertension after transplantation.

MATERIALS AND METHODS

Patients

Seventy-three patients (19 female and 54 male, with a median age of 59.5 years [23–74 years]) at 5 to 10 years after HTx were included in the prospective observational study. The median time posttransplantation at inclusion

From the Division of Transplantation,^a Department of Surgery, and Department of Internal Medicine,^b Medical University Graz, Graz, Austria.

Disclosures: none.

Received for publication Dec 5, 2009; revisions received Feb 14, 2010; accepted for publication March 20, 2010; available ahead of print May 6, 2010.

Address for reprints: Daniela Kniepeiss, MD, Department of Surgery, Division of Transplantation, Medical University, Auenbruggerplatz 29, 8036 Graz, Austria (E-mail: daniela.kniepeiss@medunigraz.at).

0022-5223/\$36.00

Copyright © 2010 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2010.03.031

Abbreviations and Acronyms

AUROC	= area under the receiver operating characteristic
CNI	= calcineurin inhibitor
EMB	= endomyocardial biopsy
GFR	= glomerular filtration rate
HTx	= heart transplantation
MDRD	= Modification of Diet in Renal Disease
MMF	= mycophenolate mofetil
ROC	= receiver operating characteristics
sCr	= serum creatinine

was 89 months (61–119 months), and the median follow-up was 48 months. Indications for HTx were dilative (78.1%) and ischemic (21.9%) cardiomyopathy. Patients with renal dysfunction requiring kidney transplantation were excluded from the analysis.

Immunosuppression

During the first week after HTx, all patients received induction therapy with antilymphocyte globulin (Lymphoglobulin, Pasteur Merieux, Leimen, Germany). Calcineurin inhibitors (CNIs) and mycophenolate mofetil (MMF) were initiated on days 2 to 4, and standard trough levels were intended to be reached on day 7. Aprednisolone was given at 15 mg/d and tapered after 6 months.

For maintenance therapy, all patients received triple drug immunosuppression consisting of a CNI, MMF, and aprednisolone. Cyclosporine trough levels were intended to be between 100 and 150 ng/mL, tacrolimus trough levels were between 3 and 8 ng/mL, and MMF levels were approximately 1 μ g/mL. Twenty patients were administered everolimus in combination with cyclosporine and aprednisolone. The indications for switching to everolimus have been described.¹² Everolimus was administered in patients with blood levels between 3 and 8 ng/mL. Cyclosporine levels in combination with everolimus were between 70 and 90 ng/mL.

Rejection Monitoring

Endomyocardial biopsies (EMBs) were performed weekly during the first month and monthly during the first 6 months after HTx. In stable HTx recipients, EMBs were performed once per year. In cases of clinical deterioration, EMB was part of our diagnostics. The results of EMBs were classified according to the International Society of Heart and Lung Transplantation guidelines. Rejections greater than grade 2 were treated with steroids or polyclonal antibodies.

Monitoring of Kidney Function

Patients were monitored after HTx on an outpatient basis, and among other routine laboratory parameters, sCr, cystatin C, and GFR, calculated by the Modification of Diet in Renal Disease (MDRD) formula,¹³ were determined.

sCr levels were determined using the Jaffe-Method, and MDRD equation was calculated following the formula $186 \times (\text{sCr} [\text{mg/dL}]^{-1.154}) \times \text{age}^{-0.203}$ in male patients and $186 \times (\text{sCr} [\text{mg/dL}]^{-1.154}) \times \text{age}^{-0.203} \times 0.742$ in female patients every 3 months. For cystatin C measurement, an automated homogeneous immunoassay using latex or polystyrene particles coated with cystatin C-specific antibodies based on nephelometry (particle-enhanced nephelometric immunoassay; Dade Behring, Marburg, Germany) was used exactly as described by the manufacturer.¹⁴

Evaluation of Risk Factors for Kidney Dysfunction

Risk factors for renal dysfunction were evaluated. As preoperative risk factors, the appearance of preexisting renal failure, hypertension, and diabetes mellitus were analyzed. The peri- and postoperative factors were use of renal replacement therapy, episodes of treated rejection, septic episodes, and use of nephrotoxic antimicrobials. Long-term factors were the presence or development of hypertension, diabetes mellitus or hyperlipidemia, clinical episodes of urinary tract infection, immunosuppressive therapy with dosage and trough levels, and duration of survival after transplantation.

Definition of Renal Impairment

Renal impairment was defined using the GFR calculated by the MDRD according to the Kidney Disease Outcomes Quality Initiative guidelines.¹⁵ A cutoff level less than and more than 30 mL/min/1.73 m² was used.^{16,17}

Statistical Analysis

All data are presented as median and interquartile range, unless otherwise noted. Data were tested for normality by the Kolmogorov–Smirnov test and the Shapiro–Wilks test. For comparison of 2 groups, continuous variables were analyzed by Student *t* test or *U* test. Binary logistic regression models were used to assess the influence of GFR calculated by the MDRD formula and serum cystatin C on renal function at 24 and 48 months after HTx. Receiver operating characteristic (ROC) curve analysis was used to express the diagnostic accuracy of cystatin C and sCr for renal function. The Youden index was used to determine the best cutoff for the evaluated parameters. The ROC curves were compared using the difference between the area test.¹⁶ An overall statistical analysis was performed. Data were evaluated using the Statistical Package for the Social Sciences version 15.0 (SPSS Inc, Chicago, IL). Youden index and ROC curve comparison were done using MedCalc version 10.0 (MedCalc Software, Mariakerke, Belgium).

RESULTS

In total, all 73 patients were analyzed. Indications for HTx and all baseline characteristics at study entry are listed in Table 1. Data were divided according to gender and are given as median and interquartile range. All factors possibly influencing the onset of renal failure are given. As can be seen from Table 1, there were no statistically significant differences. GFR calculated according to the MDRD formula differed in both groups, but the difference was not statistically significant. For immunosuppression, 87.8% of patients received cyclosporine, 61.1% of patients received everolimus, 62.2% of patients received tacrolimus, and 11.1% of patients received MMF. Throughout the study period, dialysis-dependent renal failure developed in 7 patients.

The median sCr level at the study start was 0.9 (0.8–1.3) mg/dL, the median GFR calculated by the MDRD formula was 72.8 (54.9–84.3) mL/min/1.73 m², and the median cystatin C level at baseline was 1.4 (0.88–1.83) mg/dL.

Figure 1 shows the percentage of patients with normal renal function according to all 3 measurements compared at each time point. sCr and GFR calculated according to the MDRD are simulating normal kidney function in 47%, 48%, and 36% of patients and 79%, 82%, and 74% of patients, respectively, at all time points, whereas serum cystatin C is normal in only 11% of the patients at study start, 9% of patients after 24 months of the observation period, and 6.5% of patients at study end.

TABLE 1. Baseline characteristics according to gender

	Male (n = 54)	Female (n = 19)	P value
Age (median, range)	62 (49–68)	65 (60–69)	NS
Indications for HTx (%)			
Dilated cardiomyopathy	77.6	80	NS
Ischemic cardiomyopathy	22.4	20	NS
Months after transplantation	111 (51–118)	86 (84–111)	NS
Antilipemics at study start (%)	81.6	92.3	NS
Antihypertensive medication study start (%)	85.7	84.6	NS
Diabetes at study start (%)	32.71	38.6	NS
sCr study start (mg/dL)	1.7 (1.2–2.3)	1.2 (1.1–1.9)	NS
GFR at study start (mL/min/1.73 m ²)	42.8 (32.3–67.5)	60 (37.4–61)	NS
Cystatin C at study start (mg/dL)	1.9 (1.4–2.7)	1.8 (1.4–2.6)	NS
New onset of dialysis during the study (%)	4 (9)	3 (16)	NS

Data are presented as median and interquartile range. HTx, Heart transplantation; sCr, serum creatinine; GFR, glomerular filtration rate; NS, not significant.

All included patients survived the peri-interventional period. Three patients died during the observation period (1 suicide, 2 infections). Six patients had rejections during the observation period. All rejections were managed with antithymocyte globulin (Lymphoglobulin) and steroid therapy.

Regression analysis yielded that preexisting hypertension and existing diabetes mellitus at the study start were the only risk factors that showed a significant ($P = .008$) impact on the development of renal impairment after HTx during the observation period. There was no significant impact of gender on impaired kidney function.

Everolimus as a immunosuppressive agent did not significantly influence renal function at the study end point. By

FIGURE 1. Comparison of the courses of the 3 prognostic systems. sCr and GFR calculated by the MDRD formula are seen to simulate normal kidney function for more than half of the patients at all time points. MDRD, Modification of Diet in Renal Disease.

taking all other immunosuppressive agents into account, statistical analysis revealed no significant impact of immunosuppression on the incidence of chronic renal failure ($P > .05$).

sCr, GFR calculated by the MDRD formula, and cystatin C were analyzed and compared with respect to sensitivity and specificity. The statistical results are shown in Table 2. At both time points, cystatin C showed clear prognostic significance with a P value of less than .05, whereas GFR calculated by the MDRD formula did not show significant prognostic validity. Serum cystatin C levels and sCr levels were strongly correlated at the beginning ($r = .859$, $P = .0001$) and end ($r = .886$, $P = .0001$) of the study (Figure 2).

Multivariate Analysis for Predictive Value of Cystatin C

Parameters that were considered as independent predictors for renal impairment after HTx were used for a logistic regression analysis. This revealed cystatin C measured at the study start as the superior independent predictor ($P = .0001$) for renal failure at 24 and 48 months after HTx for the whole patient set.

Cystatin C measured at the study start showed a superior diagnostic accuracy to detect renal impairment as defined by the GFR calculated according to the MDRD equation 24 and 48 months thereafter (area under the receiver operating characteristic [AUROC] 24 months = 0.96; AUROC 48 months = 0.95) (Figure 3) in the whole patient set compared with the sCr measured at the study start (AUROC 24 months = 0.89; AUROC 48 months = 0.89) (Table 3). The difference was not significant at any time point. The cystatin C level that gave the best specificity and sensitivity to detect renal impairment calculated after the Youden index for the presented patients was 0.9596. The comparison of the AUROC did not reveal a statistically significant difference between the ROC curves.

DISCUSSION

In the present study, parameters for renal dysfunction and damage of the kidney in patients after HTx were evaluated and compared. sCr is widely used as a marker for renal function, but it depends on various nonrenal factors, and major changes will occur late in the course of progressive renal impairment. In HTx recipients, sCr is even less accurate because this population has a reduced muscular mass. Moreover, chronic therapy with corticosteroids may impair this situation.¹⁷ The clinical use of cystatin C has been studied, and a significant superior sensitivity of cystatin C in comparison with creatinine was shown.⁸ Studies of cystatin C in HTx recipients are rare, and because of rather small cohorts, the results were not encouraging.¹⁸ In contrast, the results of our study confirmed cystatin C as an early prognostic marker of patients with renal dysfunction after HTx for a relatively large patient cohort.

TABLE 2. Prognostic value of serum creatinine and serum cystatin C

Time point	Parameter	P value	OR	CI 95%	CI 95%
Study start	sCr	.119	1.07	0.98	1.155
	Cystatin C	0	60011090	2965.68	1.21434E+12
24 mo after study start	sCr	.469	0.98	0.92	1.04
	Cystatin C	0	813.04	21.31	31014.23
48 mo after study start	sCr	.451	0.97	0.91	1.04
	Cystatin C	.005	1345.08	8.79	205818.46

At each time point, cystatin C demonstrated a prognostic significance ($P < .05$), whereas sCr had no influence on the development of renal failure. *CI*, Confidence interval; *OR*, odds ratio; *sCr*, serum creatine.

SCr, GFR calculated by the MDRD formula, and cystatin C were analyzed and compared with respect to sensitivity and specificity. At all time points, cystatin C showed a clear prognostic significance with a P value of less than .05, whereas sCr and GFR calculated by the MDRD formula did not show significant prognostic validity. Only 50% of the evaluated patients (eg, only 50% of long-term HTx recipients with high risks for renal impairment) were classified correctly by conventional measurements regarding their kidney function. Regarding cystatin C, only 11% of the patients had normal kidney function at the start of the study, which worsened during the course of the study (Figure 1).

Causes for renal dysfunction after transplantation are multifactorial, but CNI-associated renal impairment and renal failure have been recognized since the early days of its use. Renal dysfunction during treatment with CNI can be divided into acute and chronic forms.¹⁹ Acute toxicity is often associated with high blood levels of the drugs and is mainly observed in the early postoperative time. This type is characterized with a sudden increase in creatinine level and is in

general reversible after dose reduction. In cases of early chronic CNI-induced nephrotoxicity, dose reduction is essential and can ameliorate chronic nephropathy. Therefore, the early detection of renal complications and the possibility of identifying patients with a higher risk for renal complications are important steps to avoid progressive renal failure. Cystatin C may be able to detect renal deterioration very early, so that reversibility is still an option.

Chronic CNI nephrotoxicity occurs in the later course after transplantation and is often irreversible after dose reduction because of advanced morphologic changes of the kidneys.²⁰ Myers and colleagues²¹ evaluated the CNI-related renal structural and functional changes of kidneys in patients after transplantation. A progressive decrease in GFR, a concomitant decrease in renal plasma flow, and an increase in renal vascular resistance associated with systemic hypertension were described in the beginning. Renal biopsies presented glomerulosclerosis, striped interstitial fibrosis, and afferent arteriolopathy. In cases of irreversible CNI nephrotoxicity, the evaluation and therapy of other

FIGURE 2. There is a strong correlation ($r = 0.886$; $P = .0001$) between sCr and serum cystatin C after the 48-month observation period.

FIGURE 3. Serum cystatin C shows a high sensitivity and specificity (AUROC = 0.96) with a confidence interval of 0.915 to 1.010 and a significance of $P = .0001$ for the long-term prognosis of renal failure after HTx. AUROC, Area under the receiver operating characteristic; CI, confidence interval.

risk factors are crucial to avoid progressive renal impairment. Also, the detection of renal deterioration at an early stage is essential and cystatin C could contribute.

In regard to the dose of CNI, the correlation to renal function is still controversial. Consensus that the drug is responsible for renal failure exists, and irreversible renal damage has been reported even with low doses of cyclosporine.²² Several studies have suggested that CNI nephrotoxicity could result from individually determined susceptibility.^{3,23} Although most studies did not show a correlation between CNI levels and renal impairment in patients after HTx, it is generally agreed that efficient monitoring of cyclosporine blood levels and renal function is substantial in maintaining renal function.

Because of the high risk of organ rejection and loss of graft with changes in immunosuppression, conversion strategies are usually performed at a late time point when renal

failure is irreversible. New immunosuppressive agents such as everolimus offered the possibility to change the management of CCI therapy. Zuckermann and Aliabadi²⁴ evaluated CNI minimization protocols in HTx recipients and approved the protocols as possible and safe. Prospective randomized studies are necessary to confirm these results and to assess their influence on chronic renal failure. Our study showed that an immunosuppressive regimen with everolimus had no influence on renal impairment. We suppose that the lowest CNI target levels were not reached in this cohort and that further benefit of CNI withdrawal may be possible. Even if a direct correlation between renal impairment and CNI-free immunosuppressive therapy was not confirmed in this study, we observed that every patient whose immunosuppressive therapy was switched because of elevated cystatin C levels stabilized in terms of renal function throughout the whole time period studied. Ojo²⁵ showed that 1.5% to 2% of HTx recipients per year newly develop chronic renal failure requiring hemodialysis. This was not true for our patient cohort, possibly because of the early switch in immunosuppression as a result of cystatin C-based monitoring of kidney function.

In addition to CNI-associated nephropathy, there are many causes of renal impairment. Hamour and colleagues²⁶ emphasize that chronic kidney disease after HTx is not influenced by a CNI regimen but by several other risk factors. Renal disease before transplantation, perioperative hemodynamic insults to the kidneys, nephrotoxic effects of other drugs, dyslipidemia, hypertension, and diabetes mellitus can all contribute to chronic renal failure in recipients of non-renal organs.²⁶ The results of our study revealed pretransplant hypertension and diabetes mellitus as risk factors for the development of chronic renal failure after transplantation. Adequate therapy needs to be administered subsequently.

Plasma cystatin C levels have recently been suggested as markers for cardiovascular mortality or renal failure subsequent to cardiovascular morbidity or heart surgery.^{27,28} Cystatin C has even been proposed as an early biomarker for the prediction of possible renal failure in patients undergoing bypass grafting.²⁹ This clearly confirms our results of cystatin C as an early prognostic marker for renal failure after HTx.

CONCLUSIONS

Preventive measures to avoid progressive decline in renal function should be taken at an early stage. We suggest that cystatin C be investigated regularly in patients after HTx to recognize renal dysfunction in time and to prevent chronic renal failure. After the detection of a potential risk for patients to develop chronic kidney disease after HTx, a switch of immunosuppression and further measurements (eg, inulin plasma clearance) should be performed. Nevertheless, further randomized prospective trials are needed to confirm the role of cystatin C in patients after HTx.

TABLE 3. Diagnostic accuracies assessed by receiver operating characteristic analysis of cystatin C and serum creatinine measured at study start regarding renal function 24 and 48 months after study start

	AUROC (CI, 95%)	Range
sCr at study start to RI 24 mo	.87	0.610–1.096
Cystatin C at study start to RI 24 mo	.93	0.865–0.990
sCr at study start to RI 48 mo	.88	0.781–0.974
Cystatin C at study start to RI 48 mo	.96	0.916–1.005

AUROC, Area under the receiver operating characteristic; CI, confidence interval; sCr, serum creatinine; RI, renal insufficiency.

References

1. Wilkinson AH, Cohen DJ. Renal failure in the recipients of nonrenal solid organ transplants. *J Am Soc Nephrol*. 1999;10:1036-44.
2. Goldstein DJ, Zuech N, Sehgal V, Weinberg AD, Drusin R, Cohen D. Cyclosporine-associated end-stage nephropathy after cardiac transplantation. *Transplantation*. 1997;63:644-68.
3. Satchithananda DK, Parameshwar J, Sharples L. The incidence of end-stage renal failure in 17 years of heart transplantation: a single center experience. *J Heart Lung Transplant*. 2002;21:651-7.
4. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, et al. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med*. 2003;349(10):931-40.
5. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int*. 1985;28:830-8.
6. Skluzacek PA, Szwec RG, Nolan CR, Riley DJ, Lee S, Pergola PE. Prediction of GFR in liver transplant candidates. *Am J Kidney Dis*. 2003;42(6):1169-76.
7. Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C 8g-trace. *Scand J Clin Lab Invest*. 1985;45:97-101.
8. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V. 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.
9. Jacobsson B, Lignelid H, Bergerheim US. Transthyretin and cystatin C are catabolised in proximal tubular epithelial cells and the proteins are not useful as markers for renal cell carcinomas. *Histopathology*. 1995;26:559-64.
10. Hayashi T, Nitta K, Hatano M, Nakauchi M, Nihei H. The serum cystatin C concentration measured by particle-enhanced immunonephelometry is well correlated with inulin clearance in patients with various types of glomerulonephritis. *Nephron*. 1999;82:90-2.
11. Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. *Clin Chem*. 1998;44:1535-9.
12. Schweiger M, Wasler A, Prenner G, Stiegler P, Stadlbauer V, Schwarz M, et al. Everolimus and reduced cyclosporine trough levels in maintenance heart transplant recipients. *Transplant Immunol*. 2006;16:46-51.
13. Levey AS, Bosch JP, Lewis JP, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130:461-70.
14. Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II). *Clin Chem*. 1997;43:1016-22.
15. Patell SS, Kimmel PL, Singh A. New clinical practice guidelines for chronic kidney disease: a framework for K/DOQI. *Semin Nephrol*. 2002;22:449-58.
16. Storey JE, Rowland JT, Basic D, Conforti DA. A comparison of five clock scoring methods using ROC (receiver operating characteristic) curve analysis. *Int J Geriatr Psychiatry*. 2001;16:394-9.
17. Mariat C, Alamartine E, Afiani A. Predicting glomerular filtration rate in kidney transplantation: are the K/DOQI-guidelines applicable? *Am J Transplant*. 2005;5:2698-703.
18. Hojs R, Bevc S, Ekart R. Serum cystatin C based equation compared to serum creatinine based equations for estimation of glomerular filtration rate in patients with chronic kidney disease. *Clin Nephrol*. 2008;70:10-7.
19. Delanaye P, Nellessen E, Cavalier E, Depas G, Grosch S, Defraigne JO, et al. Is cystatin C useful for the detection and the estimation of low glomerular filtration rate in heart transplant recipients? *Transplantation*. 2007;83:641-4.
20. Greenberg A, Thompson ME, Griffith BJ. Cyclosporine nephrotoxicity in cardiac allograft patients: a seven-year follow-up. *Transplantation*. 1990;50:589-93.
21. Myers BD, Sibley R, Newton L. The long-term course of cyclosporine-associated chronic nephropathy. *Kidney Int*. 1988;33:590-600.
22. Herlitz H, Lindelöw B. Renal failure following cardiac transplantation. *Nephrol Dial Transplant*. 2000;15:311-4.
23. Van Gelder T, Balk AH, Zietse R, Hesse C, Mochtar B, Weimar W. Renal insufficiency after heart transplantation: a case-control study. *Nephrol Dial Transplant*. 1998;13:2322-6.
24. Zuckermann AO, Aliabadi A. Calcineurin-inhibitor minimization protocols in heart transplantation. *Transplant Int*. 2009;22:78-89.
25. Ojo AO. Renal disease in recipients of nonrenal solid organ transplantation. *Semin Nephrol*. 2007;27:498-507.
26. Hamour IM, Omar F, Lyster HS, Palmer A, Banner NR. Chronic kidney disease after heart transplantation. *Nephrol Dial Transplant*. 2009;24:1655-62. 2009 Jan 23 [Epub ahead of print].
27. Taglieri N, Fernandez-Berges DJ, Koenig W, Consuegra-Sanchez L, Fernandez JM, Robles N, et al., SIESTA Investigators. Plasma cystatin C for the prediction of 1-year cardiac events in Mediterranean patient with non-ST elevation acute coronary syndrome. *Atherosclerosis*. 2010;209:300-5. 2009 Sep 20 [Epub ahead of print].
28. Patel PC, Ayers CR, Murphy SA, Peshock R, Khera A, se Lemos JA, et al. Association of cystatin C with left ventricular structure and function: the Dallas Heart Study. *Circ Heart Fail*. 2009;2:98-104.
29. Wang QP, Gu JW, Zhan XH, Li H, Luo XH. Assessment of glomerular filtration rate by serum cystatin C in patients undergoing coronary artery bypass grafting. *Ann Clin Biochem*. 2009;46(Pt 6):495-500.