Evaluation of Atherosclerosis in Renal Transplanted Patients by Non-Invasive Methods

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Summary

Background: Endothelial dysfunction can be considered an early atherogenic event.

Objective: To assess atherosclerosis in renal transplanted patients through the coronary calcium score, carotid duplex scan and brachial reactivity through ultrasonography.

Methods: We assessed 30 renal transplanted male patients with stable renal function, with a mean age of 41.3 years.

Results: The detection of the atherosclerotic load in this population was very significant when the brachial reactivity technique was used (86.7%); it was less frequent when based on the presence of carotid plaque (33.3%) or the coronary calcium score (20%). The carotid plaque was considered when the thickness was > 12 mm. The coronary calcium score was abnormal when > 80 according to the Agatston scale, being observed in a low percentage of patients (21.7%), possibly due to the fact that the tomography is not the ideal method to detect atherosclerosis in renal patients, as it does not differentiates intimal calcifications of the medial layer. The adequate clinical control, the low age range and the factors related to the time of pre-transplant dialysis or the anti-inflammatory effect of the post-transplant drugs can delay the onset of the calcifications.

Conclusion: The evaluation of the atherosclerotic load through the carotid duplex scan (33,3%) and the coronary calcium score (20%) was not frequent; there was no correlation with the high rate of endothelial dysfunction detection observed with the brachial reactivity assessment (86.7%). **(Arq Bras Cardiol 2009;92(5):339-344)**

Key words: atherosclerosis; transplants/kidney; endothelium/phisiopathology.

Introduction

The vascular endothelium (VE) is a physical barrier that separates the blood from the tissues, capable of releasing vasoconstrictor and vasodilating substances, maintaining the vascular homeostasis[1].

The endothelial dysfunction (ED) can be understood as a disequilibrium between the factors that relax and constrict the vascular endothelium. The ED can be seen as an early atherogenesis event, being considered essential in the physiopathology of cardiovascular events and in the atherosclerotic process[2]. Several tests can be used aiming at the evaluation of the endothelial function (EF) and the early detection of atherosclerosis[3].

The evaluation of atherosclerosis must be initiated by the physical examination and then complemented by specialized tests, such as the assessment of the cervical and peripheral arteries and the study of the coronary calcium through a multi-detector row chest computed tomography (MDCT)[4].

The investigation of the carotid atherosclerosis uses the vascular color-flow duplex scan (DSC) aiming at the detection of carotid plaques[5,6]. The evaluation of the coronary calcium score (CCS) performed through MDCT is another effective cardiovascular disease investigation method in asymptomatic patients. The normal CCS is zero and four categories of calcification are established: 1 to 10 = minimal; 11 to 100 = mild; 101 to 400 = moderate and > 400 = significant. Finally, among the methods of early atherosclerosis assessment, one must cite the brachial reactivity (BR) assessment technique, performed by ultrasound. This method was described by Celemajer et al[3], and it is based on the capacity of the vessels to respond to a mechanical stimulus, regulate their tonus and redistribute their flow after the cessation of the stimulus, inferring the degree of endothelial dysfunction of the vessel[7].

This is a non-invasive test[3] that evaluates the percentage of dilation of the brachial artery before and after clamping with a blood pressure cuff (Aneroid Sphygmomanometer). When

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this percentage is > 10%, it is considered normal, i.e., the endothelial function is preserved. There are current directives with the technique normalizations[7]. However, few studies have comparatively investigated the different methods of assessment of early atherosclerosis.

The objective of this study was to evaluate the atherosclerotic load of renal transplanted patients through CCS, the carotid DSC and BR through ultrasound.

Methods

Thirty male renal transplanted patients were randomly selected, all with stable renal function; seven had received cadaver kidneys and the others received kidneys from living donors. Mean age was 41.3 years (ranging from 19 to 59 years) and the body mass index was estimated as ranging from 30 to 35 in 53.3% of the patients, with the remaining presenting a BMI < 30. All the study participants had more than 6 months post-transplant, to exclude the cases of acute rejection. Table 1 summarizes the clinical and laboratory data of the studied population. All patients were submitted to the following tests: carotid DSC to assess carotid plaques, chest MDCT to determine the CCS and the BR test to evaluate EF.

The carotid DSC was carried out to evaluate the presence of plaques[8]; the latter was considered when the thickness of the carotid plaque was > 12 mm and both carotids were analyzed as extensively as possible.

The chest CT was carried out in a Somaton plus volume zoom (Siemens, Germany) equipment, with four detectors, and the cutoff was established as abnormal when > 80 at the Agatston scale, based on the review article on atherothrombosis by Moreno and Furster[9], which demonstrated that CCS > 80, when associated to the intermediate group at the Framingham's scale, increased the pre-test probability of mortality of 10% in 10 years to 27%. It must be remembered that this test does not use any type of contrast.

The BR technique used the same echocardiogram device employed for the carotid duplex scan, in addition to cuffs for arterial compression, electrodes and ultrasound conducting gel, following the directives by American College of Cardiology[7]. All patients had been fasting for at least 8 hours. Regarding the technical considerations of the test, a cuff pressure of 50 mmHg above the maximum arterial pressure was used for a period of 5 minutes. Blood pressure was measured manually. Only one case had a functioning arteriovenous fistula and the contralateral arm that did not have the fistula was used in the assessment. There were no significant complications, except for complaints of numbness sensation in the examined arm, which was reported by all individuals submitted to the examination and that completely disappeared after the cuff was deflated. Six patients reported headaches after the use of sublingual nitrate and only one individual needed dipyrone for headache relief.

All study participants signed the free and informed consent form; seven patients refused to undergo the tomography scan for CCS measurement. The study was approved by the Ethics Committee in Research of our institution. The statistical analysis was carried out using descriptive measures for sample characterization, such as means, standard deviations, minimum and maximum values. The evaluation of endothelial dysfunction was carried out through the McNemar's test and significance level was set at p < 0.05.

Results

The detection of the atherosclerotic load in this population of renal transplanted patients was significant when the brachial reactivity technique was used (86.7%) and less frequent when based on the presence of carotid plaque (33.3%) or the coronary calcium score (20%).

Table 2 shows that the mean increase in the brachial artery diameter after the flow-mediated vasodilation is low (6.4%), which demonstrates endothelial dysfunction; in healthy patients, values > 10% are expected

Chart 1 demonstrates that most of the patients studied through the BR technique present a percentage of variation of the arterial diameter < 10%.

Chart 2 shows the assessment of atherosclerosis in renal transplanted individuals, comparing the BR, Carotid Plaque and CCS methods.

Discussion

The present study evaluated the atherosclerotic load of renal transplanted patients through the coronary calcium score, the carotid duplex scan and the brachial reactivity through ultrasound.

Atherosclerosis is a systemic, many times silent and life-threatening disease and its risk reduction is cost-effective[10]. A careful stratification is necessary, which allows the identification of groups at higher risk for the disease. Framingham's score is the one that is usually recommended and used, which is quite simple and easy to apply. However, it presents some limitations[11,12]. In an attempt to identify the individuals considered to be at moderate risk, those with a probability of 10% of having a cardiovascular event within 10 years, some tests have been used such as the carotid DSC, the CCS and the ischemia induced by provocative tests. It is important to stress that there are no established directives for this intermediate risk group[13] and that the selection of tests must be careful and individualized, taking into account the present risk factors and the family history data.

Chambless et al[14] demonstrated that the decrease in BP and cholesterol levels and the control of smoking habits delay the evolution of atherosclerosis and the incidence of coronary events. The ideal marker of atherosclerosis must be sensitive, preferably non-invasive, correlate with the cardiovascular event outcome, have good specificity and allow the differentiation between the presence and the absence of cardiovascular disease[15].

The measurement of the carotid intimal thickening has been established as an excellent marker in atherosclerosis detection. Currently, it is recommended that the assessment take into account the age range, the sex and ethnicity, so that a parallel between the chronological age and the vascular age can be established[16,17].

The best cutoff for the CCS is yet to be determined, but values > 400 have high specificity and correlation with lesions

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Table 1 - Clinical and laboratory data of 30 renal transplanted patients

Variables	Descriptive statistics						
	Mean	SD	Minimum	1st quart	Median	3rd quart	Maximum
Age (yrs)	41.3	10.1	19.0	35.5	41.0	49.3	59.0
BMI (kg/m^2)	25.3	4.6	19.1	21.8	24.1	27.8	38.8
Glucose	97.9	22.9	58.0	82.5	93.5	116.3	149.0
Urea	39.3	13.1	19.3	30.0	34.8	46.5	70.6
Cystatin C	1.35	0.30	0.90	1.16	1.32	1.60	2.08
PCR-T	8.06	26.26	0.13	0.68	1.67	4.28	144.20
Total cholesterol	191.9	45.6	125.0	165.5	183.0	210.3	367.0
Triglycerides	182.4	104.6	68.0	124.0	147.0	224.8	528.0
HDL	44.1	8.6	32.0	37.5	43.0	48.3	64.0
LDL	110.3	31.1	49.0	89.8	106.5	131.0	197.0
Sodium	140.2	2.0	137.0	138.8	140.0	141.3	145.0
Potassium	4.42	0.40	3.70	4.10	4.35	4.70	5.70
Uric acid	6.47	1.20	4.40	5.85	6.30	7.20	9.40
Clearance	61.5	24.2	24.7	42.7	58.7	80.2	117.1
RBC	5.17	0.82	3.33	4.71	4.93	5.65	7.40
Hemoglobin	14.8	2.1	9.9	13.5	14.2	15.9	21.0
Hematocrit	44.9	6.4	31.5	41.6	43.3	47.9	63.9
Von Willebrand (%)	80.5	81.6	10.0	13.4	46.5	121.4	357.6
Pro-BNP	314.1	453.6	10.6	55.7	148.4	292.0	2089.0
Interleukin 6	3.20	4.60	0.40	1.00	1.20	3.78	24.60
Intimal thickness	0.70	0.21	0.40	0.50	0.60	0.90	1.10
Kidney size	11.7	1.0	9.3	11.0	11.8	12.4	13.4
Anast flow	92.5	29.9	51.0	72.0	90.0	101.8	181.3
IR	0.62	0.05	0.51	0.60	0.62	0.65	0.76
Calcium	77.0	179.8	0.0	0.0	0.0	28.6	750.0
Mean BP	107.0	20.0	73.3	93.3	106.7	117.5	153.3

Table 2 – Descriptive statistics of the diameter of the brachial artery and post-ischemia variation in male renal transplanted patients (n=30)
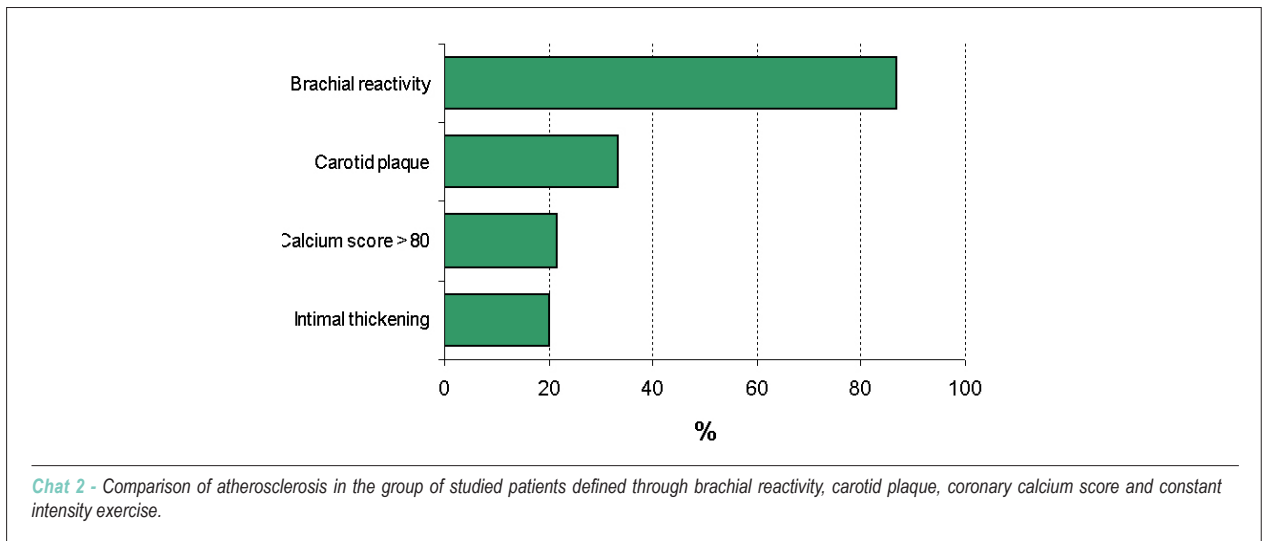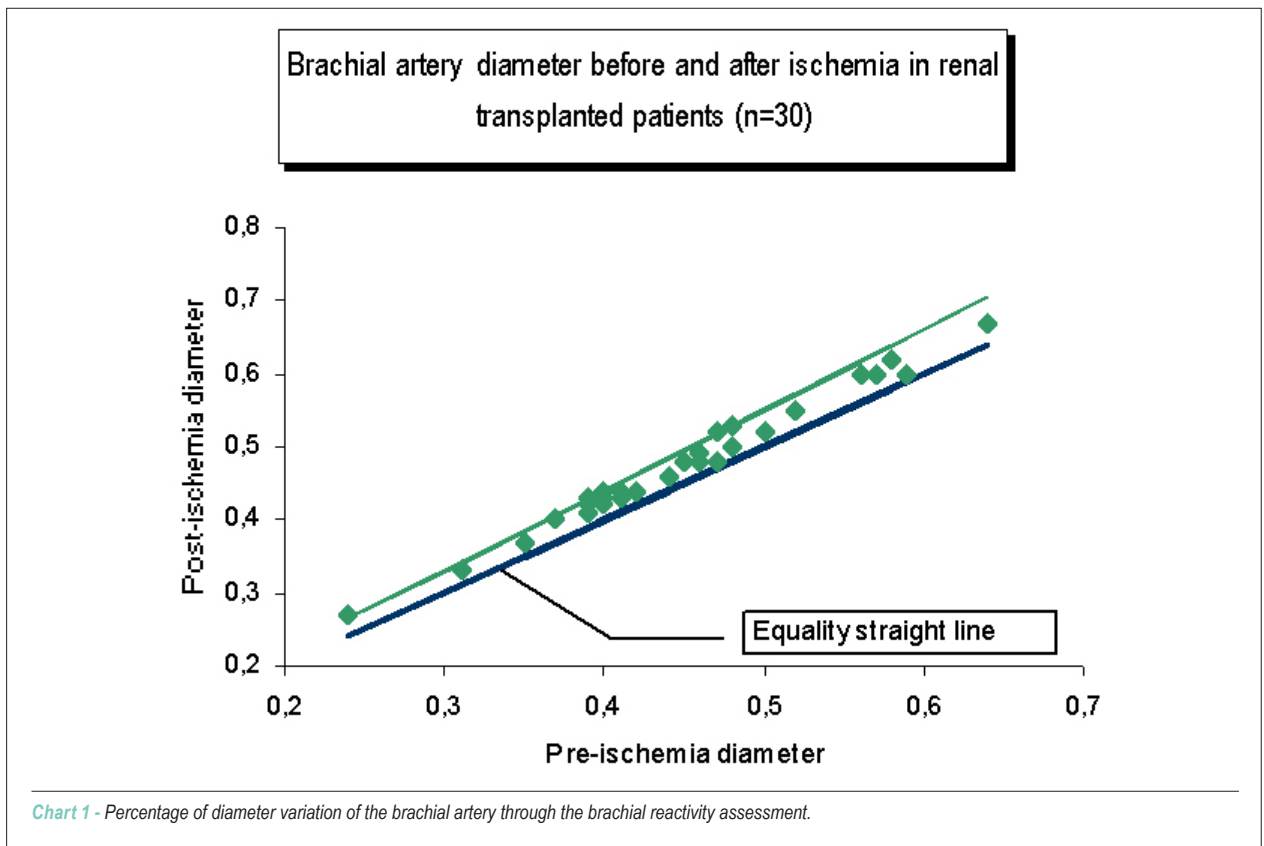
	Mean	SD	Minimum	Maximum
Pre-ischemia diameter (mm)	0.45	0.09	0.24	0.64
Post-ischemia diameter (mm)	0.47	0.09	0.27	0.67
Variation (%)	6.4	2.5	1.7	12.5

Obs: mean percentage variation for 22 healthy "controls" = 11.9%.

> 70% of coronary obstruction. Newman et al[4] evaluated 414 individuals with a mean age of 79 years and observed that 38% of them had CCS > 400. Additionally, they demonstrated that 17% of these patients would be considered normal when a combination of three other methods (ECG, ankle-arm index and constant intensity exercise) was used. Even at advanced ages there is a correlation between coronary calcification and acute myocardial infarction[18].

In the present study, the CCS was considered abnormal when > 80 at the Agatston scale, being observed in 5 of 23 (21.7%) of the studied patients. This percentage is relatively low and some possibilities must be mentioned. The first one is that the tomography may not be the ideal method to detect atherosclerosis in renal patients due to the incapacity to differentiate intimal calcifications of atherosclerosis from medial calcifications, common in renal patients[19]. Additionally, the adequate clinical control of the studied patients might delay the onset of the calcifications. Finally, the young age range of the group (mean of 41.3 years), the absence of diabetic patients, the good control of dyslipidemia and arterial hypertension through medication use, associated to the short period of time of pre-transplant dialysis (< one year) and the possible anti-inflammatory effect of the post-transplant drugs might have been delaying the coronary calcification.

Brachial artery diameter before and after ischemia in renal transplanted patients (n=30)

Chart 1 - *Percentage of diameter variation of the brachial artery through the brachial reactivity assessment.*

Chat 2 - *Comparison of atherosclerosis in the group of studied patients defined through brachial reactivity, carotid plaque, coronary calcium score and constant intensity exercise.*

It is known that the calcification of coronary arteries occurs only in atherosclerotic arteries and that atherosclerosis is necessary, but not enough, to cause a coronary event. In renal patients, some variables can promote different degrees of coronary calcification, such as the time of the renal disease, the patients' age and the disease extension. It is yet to be determined whether a calcium/phosphorus ratio imbalance, which might be present in renal patients, could also be an extra factor that favors the increase in the CCS of these patients[20].

The BR test has been used more frequently in research than as a clinical tool. This is due to some factors, such as the fact that it is a new technique, still being validated; it is an operator-dependent test, with recently established directives that has been undergoing improvement. It is known that the endothelial

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dysfunction can precede the formation of atheroma plaques and perhaps the damage in the macrocirculation, which is evaluated by the BR technique, does not reflect the impact of the endothelial damage in other organs[21,22].

One of the main limitations of this study is the fact that it is a transversal evaluation. That is, to evaluate the subclinical atheromatosis, the time of the patients' exposition to the risk factors is an important fact. Perhaps the younger age range of the studied population (mean of 41 years) is a second limitation. Regarding the methods used, the carotid intimal thickening and the coronary calcium score should be evaluated taking into account the age range, the sex and even the ethnicity. Unfortunately, there are no Tables with these data in our country. It is also worth mentioning that the brachial reactivity test is a new, operator-dependent technique and needs a learning curve.

It is important to mention that cardiovascular disease is the most important cause of mortality in patients with advanced chronic renal failure and in those submitted to renal transplant[23] and that the detection of a significant atherosclerotic load can help in the risk stratification of this population.

In conclusion, in this series, the detection of the atherosclerotic load was more significant in the brachial reactivity test and less frequent when evaluating the presence of the carotid plaque at the duplex scan or the coronary calcium score. Considering that this is

a high-risk population, renal transplanted patients would perhaps deserve a more aggressive strategy of diagnostic investigation of the endothelia function and subclinical atheromatosis, including laboratory tests, brachial reactivity test, carotid duplex scan and coronary calcium assessment. New strategies to decrease the risk of cardiovascular events, including individualized and even more intense treatments, can decrease the incidence of cardiovascular events[24]. Further studies are necessary to confirm whether the early detection of subclinical atherosclerosis in at-risk populations will benefit from a more aggressive prevention strategy.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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References

1. Verma S, Anderson T. Fundamentals of endothelial function for the clinical cardiologist. Circulation. 2002; 105: 546-9.

2. Hermann J, Lerman A. The endothelium: dysfunction and beyond. J Nucl Cardiol. 2001; 8: 197-206.

3. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992; 30: 1111-5.

4. Newman AB, Naydeck BL, Sutton-Tyrrell K, Edmundowicz D, leary DO, Kronmal R, et al. Relationship between coronary artery calcification and other measures of subclinical cardiovascular disease in older adults. Arterioscler Thromb Vasc Biol. 2002; 22: 1674-9.

5. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women. The British Regional Heart Study. Stroke. 1999; 30: 841-50.

6. Held C, Hjemdahl P, Eriksson SV, Bjorkander I, Forslund L, Rehnqvist N. Prognostic implications of intima media thickness and plaques in the carotid and femoral arteries in patients with stable angina pectoris. Eur Heart J. 2001; 22: 62-72.

7. Corretti MC, Anderson TJ, Benjamim EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of the endothelial-dependent flow mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002; 39: 257-65.

8. Douglas PS. Using imaging for cardiovascular risk prediction: an overview. J Am Soc Echocardiogr. 2004; 17: 350-2.

9. Moreno PR, Fuster V. The year in atherothrombosis. J Am Coll Cardiol. 2004; 44: 2099-110.

10. Cohn JN, Quyyuni AA, Hollenberg NK. Surrogate markers for cardiovascular disease: functional markers. Circulation. 2004; 110 (Suppl. 4): IV31-IV36.

11. Wilson P W, D'Agostinho R B, Levy D Prediction of coronary heart disease using risk factor categories. Circulation. 1998; 97: 1837-45.

12. D`Agostinho RB Sr, Grundy S, Sullivan LM, Wilson P, CDH Risk Prediction Risk. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA. 2001; 286 (2): 180-7.

13. Greeland P, Gaziano M Selecting asymptomatic patients for coronary computed tomography or electrocardiography exercise testing. N Engl J Med. 2003; 349: 465-73.

14. Chambless LE, Folson AR, Davis V, Sharrett R, Heiss G, Sorlie P, et al. Risk factors for progression of common carotid atherosclerosis: the Atherosclerosis Risk in Communities Study, 1987-1998. Am J Epidemiol. 2002; 155: 38-47.

15. de Groot E, Hovingh K, Wiegman A, Duriez P, Smit AJ, Fruchart JC, et al. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation. 2004; 109 (23 Suppl. 1): III33-8.

16. Stein JH, Douglas PS, Srinivasan SR, Bond MG, Tang R, Li S, et al. Distribution and cross sectional age related increases of carotid artery intima media thickness in young adults: the Bogalusa Heart Study. Stroke. 2004; 35: 2782-7.

17. Stein JH. Carotid intima media thickness and vascular age: you are only as old your arteries look. J Am Soc Echocardiogr. 2004; 17: 686-9.

18. Thompson GR, Partridge J. Coronary calcification score: the coronary-risk impact factor. Lancet. 2004; 363: 557-9.

19. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. AHA Councils on Kidney in Cardiovascular Disease. High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation. 2003; 108: 2154-69.

20. McCullough PA, Soman S. Cardiovascular calcification in patients with chronic renal failure: are we on target with this risk factor? Kidney Int Suppl. 2004; 90: 518-24.

21. Kuvin JT, Karas RH. Clinical utility of endothelial function testing. Circulation. 2003; 107: 3243-7.

22. Vogel RA, Correti MC, Plotnick GD. A comparision of braquial artery flow – mediated vasodilatation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. Clin Cardiol. 2000; 23: 571-5.

23. de Souza Delgado D, Gerola LR, Hossne NA Jr, Branco JN, Buffolo E. Revascularização do miocárdio em pacientes com transplante renal. Arq Bras Cardiol. 2002; 79 (5): 476-9.

24. Gill JS. Cardiovascular disease in transplant recipients: current and future strategies. Clin J Am Soc Nephrol. 2008; 3 (Suppl 2): S29-37.