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A prospective, 3-year follow-up study of vascular function and cardiac autonomic control following renal transplantation

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A PROSPECTIVE, 3-YEAR FOLLOW-UP STUDY OF VASCULAR FUNCTION AND CARDIAC AUTONOMIC CONTROL FOLLOWING RENAL TRANSPLANTATION

by

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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CHAPTER I

INTRODUCTION

Epidemiology

Chronic kidney disease (CKD) is a worldwide public health problem. According to the National Health and Nutrition Examination Survey, CKD affects 8.3 million individuals over 20 years old in the United States, 4.6% of the total population. In the United States alone, the prevalence of end-stage renal disease (ESRD) is increasing at an alarming rate (1). The number of patients enrolled in the ESRD Medicare-funded program had increased from approximately 10,000 in 1973 to 86,354 in 1983, and to 547,982 as of December 31, 2008 (2). Although the exact reasons for the growth of the ESRD program are unknown, factors including the increased incidence of diabetes and hypertension, changes in the demographics of the population, differences in disease burden among racial groups, and under-recognition of early stages of CKD may partially explain this growth (1,3).

Patients with ESRD consume a disproportionate share of health care resources, with the total cost of the ESRD program in the United States reaching approximately $39.46 billion in 2008. Medicare costs per ESRD patient per year were nearly $66,000 overall, ranging from $26,668 for transplant patients to $77,506 for patients receiving hemodialysis therapy (2). However, despite the large magnitude of health care resources committed to the treatment of ESRD, these patients continue to experience significant mortality, morbidity, and a reduced quality of life. In the United States, approximately 300,000 patients who suffer from ESRD are receiving dialysis therapy, while only 10,000 patients per year receive a kidney transplant (4). Currently, there are 60,000 patients on dialysis treatment on the waiting list for a kidney transplant (1). Over the past five years,

the number of new patients with kidney failure has averaged more than 90,000 annually. The average ESRD patient is male (54.8%), Caucasian (60.1%), and between 45 and 64 years of age (40.9%). The leading causes of ESRD include diabetes (34.6%) and hypertension (22.9%). Glomerulonephritis (15.6%), cystic kidney disease (4.3%), other urologic disorders (1.9%), and other unknown causes (20.1%) account for the other 42% of causes of ESRD. Within the United States, 67,000 deaths occur annually as a result of kidney failure (4).

Neuropathy in ESRD

According to the National Kidney Foundation, chronic kidney failure is measured in five stages, which are calculated using a patient's glomerular filtration rate (GFR). Stage 5 (GFR<15 mL/min) or less than 10% renal function, is classified as ESRD and typically requires some form of renal replacement therapy or kidney transplant (5). ESRD occurs when nephrons are irretrievably impaired, causing the retention of metabolic waste products, salt, and water to become potentially fatal (6). The term uremia is used to describe the syndrome of manifestations the patient experiences as toxins accumulate in the plasma in the final stage of renal insufficiency and resultant multi-organ failure. Uremia is often manifested by the presentation of elevated blood urea and creatinine, accompanied by fatigue, vomiting, nausea, loss of appetite, and neurological changes (7). These neurological changes were first noted in the early 1970s, when the first clinical neurophysiological investigations in ESRD patients reported high rates of neuropathy and significant reductions in motor nerve conduction velocity, generally relating the development of neuropathy to the severity of renal failure (8, 9). Later studies confirmed these initial results, also noting a correlation between the extent

of renal impairment and the degree of neuronal conduction slowing, as well as improvement in neurophysiological parameters following renal transplantation (10,11-13). Of a particular note, studies by Nielson et al. (14) and Bolton et al. (15) provided clinical evidence to suggest that a uremic toxin (K^+) was responsible for the development of neuropathy in ESRD patients, a hypothesis that remains a major focus of neurophysiological research into this condition.

Uremic neuropathy in ESRD patients commonly presents as both peripheral and autonomic neuropathy, with autonomic neuropathy being the focus of the current study. Autonomic neuropathy is considered as part of polyneuropathy, which typically manifests itself as distal, symmetric, and both sensory-motor and autonomic dysfunction. Most of the common features of autonomic neuropathy, including post-dialysis hypotension, dizziness, sphincter dysfunction, and gastrointestinal disturbances, are not life-threatening but are debilitating. Others, such as prolongation of the electrical systole of the heart (Q-T interval) observed as heart rate decreases with hypotension, have been linked to increased risk of sudden cardiac death (16-18). Past studies have suggested that the elongation of the electrical systole of the heart may be due to an imbalance between the sympathetic and parasympathetic innervation of the heart (19). It is well known that the parasympathetic nervous system protects the heart from rhythm disturbances, especially ventricular fibrillation (20). Thus, uremic autonomic neuropathy, by disturbing the normal electrical activity of the heart via impaired parasympathetic nervous system function, can potentially cause cardiac autonomic dysfunction in ESRD patients. This cardiac autonomic dysfunction is often indicated by reduced heart rate

variability (HRV) (21). As demonstrated in a number of prominent studies, reduced HRV is closely linked to increased cardiovascular morbidity and mortality (22-24).

In conjunction with autonomic dysfunction, ESRD patients also possess additional risk factors that contribute to their greater incidence of cardiovascular events. In fact, the presence of cardiovascular disease is the most important predictor of mortality in patients with ESRD, accounting for almost 50% of deaths (25). Of these, approximately 20% can be attributed to the consequences of coronary artery disease (CAD). First, ESRD patients have an increased likelihood of possessing the traditional risk factors for cardiovascular disease, including hypertension, dyslipidemia, low serum HDL cholesterol, diabetes, left ventricular hypertrophy, and increased age. As compared to the general population, ESRD patients are 60% more likely to possess two or more of these risk factors. In addition to traditional risk factors, ESRD patients also possess several factors unique to the loss of renal function that place them at a higher risk for cardiovascular disease (26). For example, uremia and renal replacement therapies result in markedly enhanced oxidant stress, the production of cytokines, increased adhesion molecules in endothelial cells, and other proinflammatory factors that provide an environment favoring the development of accelerated atherosclerosis (27-30). ESRD patients also exhibit increased serum levels of C-reactive protein, a protein released in inflammatory disorders, which serves as a major risk factor for coronary artery calcification. The relationship between endothelial dysfunction and ESRD will be explored in detail in later sections. Lastly, in dialysis patients, increased oral intake of calcium (which is given as a phosphate binder to treat hyperphosphatemia) may also directly increase coronary artery calcifications, thus greatly increasing the risk of CAD

(31). Importantly, despite correction of several uremic parameters by renal replacement therapies, the mortality and morbidity rates remain high in ESRD patients, predominantly due to cardiovascular disease.

Heart Rate Variability: Overview

Heart rate variability (HRV) is defined by the variation of the time intervals between two consecutive heartbeats (Figure B1). There are a variety of techniques that can be used to detect heartbeats, including ECG, blood pressure wave form recordings, and the pulse wave signal derived from a photoplethysmograph. Because it provides the most accurate beat-by-beat heart rate time series, ECG is the most commonly used method for obtaining HRV (33). The first documented observation of HRV is often credited to Stephen Hales in 1773, who observed a respiratory pattern in the blood pressure and pulse of a horse (64). Approximately a century later, Carl Ludwig was able to observe a regular quickening of pulse rate with inspiration and slowing with exhalation in a dog by means of a Kymograph, a device that allowed mechanical activity to be recorded on a smoked drum. This represented the first documented report of respiratory sinus arrhythmia (RSA), and the subsequent work of Donders (1868) focused on this relationship between respiration, heart rate, and the vagus nerve (64). Early clinical research on the topic of HRV continued in 1965, when Schneider and Costiloe reported that the period of time between two consecutive heartbeats is not constant throughout a person's heartbeats (34). That same year, Hon and Lee demonstrated the first clinical relevance of HRV in fetal heart rate patterns. They noted that fetal distress was preceded by alternations in interbeat intervals before any noticeable change occurred in the heart

rate itself (35). During the 1970s, research was taken one step further with studies by Ewing et al. (36), who devised a number of simple tests including short-term RR-interval variability to test autonomic neuropathy in diabetic patients. In 1981, Akselrod et al. (37) introduced power spectral analysis of heart rate fluctuations in order to quantitatively evaluate beat-to-beat cardiovascular control. The clinical importance of HRV became fully appreciated in the late 1980s, when it was confirmed that HRV was a strong and independent predicator of mortality after an acute myocardial infarction (24,38).

These early studies and others played an instrumental role in shaping the now widely held belief that HRV can indicate the heart's ability to respond to changes in cardiac autonomic activity (33). The normal variability in heart rate (HR) is predominantly dependent on extrinsic regulation of the heart rate due to autonomic neural regulation (39,48). HR is primarily controlled by the delicate balance between the two branches of the autonomic nervous system, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The sympathetic branch, typically activated in response to stress, exercise, or heart disease, causes an increase in HR via release of norepinephrine to the sino-atrial (SA) node, which increases the firing rate of cells within the SA node. The parasympathetic branch, on the other hand, acts to decrease HR via release of acetylcholine by the cardiac branches of the vagus nerve to the SA node. The vagal and sympathetic activities of the heart constantly interact, producing characteristic rhythmic patterns of HRV (33). These characteristic HRV patterns allow HRV analysis to separate sympathetic and parasympathetic contributions to HRV, making HRV analysis a useful tool in studying the role of the autonomic nervous system in modulating HR. Because alterations in autonomic function occur in several cardiac conditions,

including myocardial infarction, congestive heart failure, and diabetic neuropathy, HRV is an invaluable tool that may allow for preventive intervention at early stages of the disease when it is most effective and beneficial (40).

Heart Rate Variability Analyses

Over the past several decades, HRV analysis has emerged as a powerful tool in the assessment of autonomic modulation of cardiac function due to its non-invasive, reproducible, and economical nature. The source data for HRV is a continuous beat-by-beat measurement of interbeat intervals, which can be obtained from single-lead chest electrocardiogram recordings (41). Chest electrocardiograms can be obtained quickly, are inexpensive, and are widely available in a variety of hospital and clinic settings, thus making HRV analyses one of the most commonly used physiological parameters for cardiovascular risk stratification (42).

There are two types of heart rate variability analyses: short- and long-term analyses. Short-term HRV, typically derived from 5-minute ECG recordings (43), are performed at steady-state physiological conditions (typically in the supine position) and are considered more practical for clinical applications of HRV assessment. However, it has been demonstrated that the predictive power of short-term recordings of HRV is significantly lower than that of long-term recordings, thus limiting the predictive value for assessment of cardiovascular diseases and risk factors by short-term HRV (44). Long-term HRV, derived from data obtained from 24-hour Holter ECG recordings, may provide a higher power of prediction of cardiovascular events, although the cost of such methods is significantly greater. Both short- and long-term HRV analyses are frequently

utilized in the clinical and research setting, and it is often the choice of the researcher or clinician as to which method best suits his/her needs for a given study (41).

In both short- and long-term ECG recordings, heart rate variability can be assessed in the time and frequency domain. Time domain analysis measures the changes in heart rate over the intervals between successive normal cardiac cycles (33,45,46). From a continuous ECG recording, each QRS complex is detected and the normal RR intervals (NN intervals) are determined (39) (See Figure B1). Normal RR intervals are considered RR intervals that arise from the sinus node and are not associated with cardiac arrhythmia. Two types of HRV indices are distinguished in time domain analysis. Beat-to-beat or short-term variability (STV) represent rapid changes in heart rate, while long-term variability (LTV) represents slower fluctuations in heart rate. Both types of indices are calculated from the RR intervals occurring in the chosen time window (33). From the original RR intervals, a number of parameters can be calculated. The simplest variable to calculate is the standard deviation of the NN intervals (SDNN), or the square root of variance. Because variance is mathematically equal to total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording. Thus, SDNN encompasses both short-term high frequency variations and short-term low frequency variations. The most commonly used statistical measures derived from interval differences include RMSSD, the square root of the mean squared differences of successive NN intervals, and SDSD, the standard deviation of differences between adjacent NN intervals. RMSSD and SDSD mainly measure the short-term high frequency variations in heart rate. Other useful parameters include SENN, the standard error of the mean, and pNN50%, the percentage of successive heart beats which differ by

more than 50 ms (46). For the purposes of this project, SDNN and RMSSD were utilized.

While time domain methods are computationally simple, they are less useful in discriminating between sympathetic and parasympathetic contributions to HRV, a role primarily performed by frequency domain analysis. In frequency domain analysis, sympathetic and parasympathetic modulation of cardiac function are assessed in the low frequency (LF) range (0.04-0.15 Hz), while parasympathetic modulation is assessed in the high frequency (HF) range (0.15-0.4 Hz) (33,47,48). Furthermore, the LF/HF ratio is commonly used as a parameter reflecting the sympathovagal balance of autonomic function. Clinically, frequency domain analysis provides a powerful tool in distinguishing between sympathetic and parasympathetic contribution to HRV in patients, thus making it important in the assessment of prognosis of a variety of pathological conditions and disease states, such as uremic autonomic neuropathy.

Vascular Endothelium

The endothelium is the thin layer of cells that lines the interior surface of blood vessels, forming an interface between the circulating blood in the lumen and the vessel wall. Endothelial cells line the entirety of the circulatory system, including the heart, arteries, veins, and capillaries (49). The strategic location of endothelial cells allows for regulation of vascular tone by responding to changes in hemodynamic forces (e.g. shear stress) and blood-borne signals through release of vasoactive substances. A critical balance between endothelium-derived relaxing factors and contracting factors serves to maintain vascular homeostasis. When this balance is disturbed, it predisposes the

vasculature to vasoconstriction, leukocyte adherence, platelet activation, coagulation, inflammation, and atherosclerosis (50).

The primary vasodilator released by the endothelium is Nitric Oxide (NO). NO plays an important role in the maintenance of vascular tone and reactivity and serves to oppose the actions of various endothelium-derived contracting factors (51). NO further acts to inhibit platelet and leukocyte activation and maintains the vascular smooth muscle in a nonproliferative state. Other relaxing factors affecting the endothelium include endothelium-derived hyperpolarizing factor, prostacyclin, C-type natriuretic factor, adenosine triphosphate (ATP), substance P, and acetylcholine (52,53). In a resting state, basal blood flow maintains a continuous release of endothelium-dependent relaxing factors, while an increase in blood flow increases this release of relaxing factors (54). This increase in release of relaxing factors is mediated by the increase in shear stress acting on the endothelium via stretch-sensitive mechanoreceptors, leading to subsequent vessel dilation and reduction in wall stress (55,65).

The endothelium is also affected by various contracting factors, such as endothelin-1 acting on ET_A receptors (ET-1), angiotensin-II, thromboxane A_2, prostaglandin H_2, and ATP (53,54). Stimuli that initiate release of these endothelium-dependent vasoconstrictors include norepinephrine, thrombin, hypoxia, and stretch (52). Normally, plasma levels of vasoconstrictors are low; however, higher levels have been reported in some diseases states, such as hypertension, although the exact role these factors play in these states remains unclear (56). Additionally, some vasoconstrictors, such as ET-1, actively participate in leukocyte and platelet activation as well as facilitate a prothrombotic and proatherogenic state within the vessel (57).

Endothelial function is typically studied by exposing blood vessels to an endothelium-dependent vasodilating stimulus and subsequently measuring the extent of vasodilation in response to the stimulus (58). This can be accomplished by a variety of different methods, the most common of which include brachial artery flow-mediated dilation (FMD) and forearm vascular resistance. Noninvasive brachial artery assessment of FMD has been utilized for measurement of endothelial function for over 15 years and has become an important research tool in measuring pre-clinical cardiovascular risk for populations with and without risk factors (59,60). FMD is the measurement of transient changes in brachial artery diameter in response to shear stress. An increase in flow in the brachial artery is caused by inflation and deflation of a pneumatic cuff on the upper arm. Upon deflation of the cuff, the increase in flow results in shear stress, which subsequently causes release of NO and vessel vasodilation. Doppler ultrasound technology is utilized to observe this change in vessel diameter, and FMD is measured as the percentage change in brachial artery diameter from baseline in response to the increased flow (61,62). Alternatively, forearm vascular resistance can also be utilized to assess endothelial function. This method uses plethysmography rather than Doppler ultrasound technology to assess flow through both conduit arteries and resistance arteries. Forearm vascular resistance can be determined as a ratio of change in blood pressure and blood flow. (62,63).

Endothelial Dysfunction in CKD

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with CKD, with a 3.5-50 times higher estimated risk for cardiovascular events in

these patients as compared to the general population (66,67). The pathophysiology of many of these cardiovascular diseases, including coronary artery disease, which represents 20% of all CKD deaths, has been attributed in part to endothelial dysfunction. Endothelial dysfunction is characterized by a disruption of the delicate balance between vasodilation, oxidative stress, inflammation, thrombosis, and fibrinolysis maintained by endothelial cells (68-70). Traditional risk factors for CVD, including aging, diabetes, dyslipidemia, hypertension, and obesity, alter the balance between these various factors, resulting in abnormal endothelial functions (71-73). In patients with CKD, traditional risk factors are unable to completely explain the increased CV event rate reported in CKD patients, and the mechanisms for this elevated CV risk are thought to involve changes in both the heart and vasculature. CKD is often associated with hypertension and left ventricular hypertrophy, which may result in sudden arrthymic death. Changes in the vasculature include endothelial dysfunction, smooth muscle cell hyperplasia/hypertrophy, vascular calcification, and atherosclerosis (74-76). Endothelial dysfunction is present not only in Stage 5 CKD but also in earlier pre-dialysis stages of CKD as well, suggesting that endothelial dysfunction plays a crucial role in mediating the increased cardiovascular risk for all CKD patients (77). Furthermore, endothelial damage and injury have been closely linked with such clinical conditions as thrombosis, hypertension, renal failure, and atherosclerosis and may be responsible for the accelerated atherosclerosis demonstrated in patients with chronic renal failure (78).

Although the exact mechanism of endothelial dysfunction in CKD patients is not completely understood, recent evidence suggests that oxidative stress, L-arginine deficiency, and asymmetric dimethlyarginine (ADMA) inhibition of NO synthesis all play

key roles in the pathogenesis of endothelial dysfunction in CKD (79). Of these potential mechanisms, oxidative stress is the most widely studied contributor to endothelial dysfunction (81). Oxidative stress is defined as the tissue damage resulting from an imbalance between the generation of oxidative compounds and removal by endogenous antioxidants (82). Generation of oxidative compounds plays an important role in inflammation and tissue repair processes, and improper activation of these oxidative processes contributes to cell and tissue injury, primarily through endothelial nitric oxide synthase (eNOS) and NO dependent pathways (83). In CKD patients, the balance between pro- and anti-oxidant factors is shifted towards increased oxidative stress. In recent studies, deficiencies in anti-oxidant defense mechanisms have been demonstrated in CKD patients, including reduced levels of vitamin E (due to dietary restrictions of fresh fruits and vegetables to avoid hyperkalemia and loss of vitamin during dialysis), reduced intracellular levels of vitamin E, reduced selenium concentrations, and deficiency of glutathione peroxidase (84-87). At the same time, pro-oxidant activity is increased. Factors contributing to increased pro-oxidant activity include characteristics of the patient population suffering from renal disease, such as advanced age and diabetes, uremia, chronic inflammation, and factors associated with renal replacement therapies. Hemodialysis may also induce repetitive bouts of oxidative stress, mainly through membrane bio-incompatibility (88,89). Although the imbalance between pro- and anti-oxidant factors begins during the early stages of CKD, it is most pronounced in patients on hemodialysis, suggesting that the onset of hemodialysis alone significantly worsens oxidative stress (80). Due to this imbalance, markers of oxidant stress, including lipid hydroperoxides, oxidized glutathione, protein carbonyls, and F2 isoprostanes are greatly

increased in CKD patients (90-92). Importantly, markers of oxidative stress are inversely correlated with endothelial function in patients with CKD, thus suggesting that oxidative stress may promote endothelial dysfunction and atherosclerosis and, therefore, cardiovascular complications in CKD patients (80). In addition, this imbalance, along with other metabolic changes in the endothelium, may lead to a low-grade inflammatory state, which further promotes an atherogenic environment and increases the risk of coronary ischemia in patients with renal insufficiency (93, 94).

Along with oxidative stress, L-arginine deficiency is also recognized as a potential mechanism for endothelial dysfunction in CKD. Within endothelial cells, nitric oxide synthesis relies on the amino acid substrate L-arginine. L-arginine synthesis primarily occurs in the proximal tubule of the renal cortex and is impaired with loss of functional renal mass, as occurs with chronic renal insufficiency (95). However, despite this reduced production, the plasma concentration of L-arginine in patients with CKD appears to be maintained at normal levels, perhaps due to increased amino acid release into the blood due to skeletal muscle wasting (96). Additionally, with the progressive loss of renal function in CKD, uremic toxins tend to accumulate in the plasma, which has been linked to inhibition of L-arginine transport into endothelial cells. This inhibition of L-arginine transport results in reduced substrate availability for NO production, leading to the decreased endothelial-dependent dilation present in CKD, while accounting for the seemingly normal L-arginine plasma levels in these patients (97). Hyperhomocysteinemia, typically present in CKD patients, has also been shown to reduce L-arginine uptake in endothelial cells and impair endothelial-dependent relaxation, which may further contribute to the uremic inhibition of L-arginine transport

present in patients with renal insufficiency (98). Whether due to accumulation of uremic toxins or hyperhomocysteinemia, this inhibition of L-arginine transport leads to reduced endothelial NO production in CKD patients, which may cause vasoconstriction and hypertension, thereby resulting in adverse cardiovascular outcomes (97).

Another potential contributor to endothelial dysfunction in CKD is the formation of the endogenous NOS inhibitors asymmetric dimethylarginine (ADMA) and *N*-monomethylarginine (L-NMMA) (99,100). Both ADMA and L-NMMA are the result of post-transcriptional methylation of L-arginine residues by protein arginine methyltransferases (PRMTs) and are released in their free form following protein hydrolysis. ADMA production is approximately 10-fold that of L-NMMA and is elevated in patients with chronic renal failure (101). Plasma levels of ADMA have been found to predict progression to ESRD in patients with CKD and ultimately predict adverse cardiovascular events in patients with mild to moderate CKD (102,103). In patients with renal damage, clearance of ADMA in the urine is impaired and contributes to elevated plasma levels of ADMA. However, decreased urinary clearance is not the primary reason for elevated ADMA in CKD, rather increased PRMT activity and expression and reduced degradation by dimethlyarginine dimethylaminohydrolase (DDAH) are likely the major causes of increased ADMA in CKD (95). ADMA is considered a uremic toxin and exhibits adverse cardiovascular effects in both CKD patients and healthy subjects. In healthy subjects infused with ADMA, heart rate and cardiac output were reduced while mean arterial pressure increased (104). ADMA has also been linked with impaired endothelial function, acting primarily via its action as a competitive inhibitor of eNOS and subsequently reducing NO production in the

the endothelium (105). Interestingly, antioxidant therapy has been shown to reduce ADMA levels in patients with CKD and may be an effective treatment strategy to restore endothelial function (106). In summary, although research into these potential mechanisms of endothelial dysfunction in CKD patients is promising, the confounding effects of other risk factors in CKD make it difficult to determine whether the true source of endothelial dysfunction is due to renal impairment or the combination of multiple risk factors in this patient population.

Hypotheses and Study Aims

This study has multiple aims:

1.) Evaluate cardiac autonomic control in patients with ESRD before and 3 months, 12 months, and 36 months following renal transplantation (RTX). Cardiac autonomic function was assessed via time-and frequency-domain HRV analysis. We hypothesize that renal transplantation will improve cardiac autonomic function in ESRD patients, which may contribute to a reduction in cardiovascular risk.

2.) Assess endothelial vascular function in patients with ESRD immediately before and 3 months, 12 months, and 36 months following RTX. Vascular function was assessed by brachial artery FMD. We hypothesized that renal transplantation improves endothelial vascular function, which may also contribute to the reduced cardiovascular risk in ESRD patients after RTX.

3.) Assess whether cardiovascular variability is correlated with vascular function before and after kidney transplantation. We hypothesize that improved endothelial vascular

function in response to RTX is associated with improved cardiac autonomic function and, therefore, reduced cardiovascular risk.

CHAPTER II

METHODS

The following is an abridgment from a grant application kindly provided by Dr. Roberto Kalil.

Subject Recruitment

Several groups of subjects were recruited for the original study, including living-donor kidney transplant recipients, cadaver-donor kidney transplant recipients, and subjects on the waiting list for a kidney transplant (control group), but only one group (Group 1) of subjects was utilized in the current study. Group 1 included living-donor kidney transplant recipients, and, for purposes of the current study, only data from Group 1 subjects was utilized. Potential subjects were recruited using mailed cover letters and consent forms explaining the study, which were distributed after a patient was formally listed to undergo a living-donor kidney transplant. Applicable names/addresses were derived from the kidney transplant waiting list from University of Iowa Hospitals and Clinics (UIHC) and Veterans Affairs Medical Center (VAMC). Attached to the cover letter was a phone number for potential subjects to call for any questions. If a phone call or a returned signed consent form was not received within two weeks after mailing the consent form, a research assistant called the potential subject to determine if he/she was interested or had any questions about the study. Written, witnessed consent was obtained for all subjects using IRB approved information forms. After consent was granted, subjects were asked to come to the Clinical Research Unit (CRU) at the University of Iowa Hospitals for study visit #1. Visit #1 was scheduled for a date preceding their living-donor kidney transplantation (range from one month to one day preceding

transplantation). The average age of the subjects was 42±4.2 years. The following inclusion criteria were utilized: age 18-99 years, male or female, and the subject must be formally listed as a living donor kidney transplant recipient. No exclusion criteria were utilized for the current study. Subjects were asked to participate in the study for approximately three years, which included four study visits. Visit #1 took place before the subject's living-donor kidney transplantation, visit #2 three months after transplantation, visit #3 one year after transplantation, and visit #4 three years after transplantation, respectively. Study visits took place in the CRU and Human Cardiovascular Laboratories at the University of Iowa Hospitals.

Data Collection

Arterial Pressure Measurement and Blood and Urine Sampling

Upon arrival to the CRU for each study visit (Visit #1-4), a subject's height, weight, and blood pressure were measured and recorded. Arterial pressure was measured in the sitting position using a mercury sphygmomanometer after the subject had been resting for approximately 5 minutes. Each arterial blood pressure measurement was taken twice for accuracy. Additional measurements were taken if the readings differed by more than 10%. Supine arterial pressure was measured in duplicate at intervals during the experimental protocols using a non-invasive oscillometric sphygmomanometer (Lifestat 200, Physio-Control, Redmond, WA). Following blood pressure measurement, two ounces of blood were drawn from a vein in the arm (median cubital) in order to gain information on possible mechanisms of atherosclerosis. Laboratory parameters determined included: asymmetrical dimethlyarginine (ADMA) levels, isoprostanes,

homocysteine, hs C-reactive protein, lipids, fibrinogen, intact parathyroid hormone (PTH), insulin, fetuin A, fibroblast growth factor 23 (FGF-23), mutations in genes encoding for extracellular superoxide dismutase (EC-SOD), and myeloperoxidase (MPO). Additionally, subjects were asked to provide a urine sample, which provided information regarding urinary isoprostanes and urinary albumin-to-creatinine ratio. In female subjects, the urine sample was also utilized for pregnancy testing. If a female subject was pregnant, she was not permitted to participate in the cardiac CT scan due to radiation exposure. Blood and urine samples were collected and analyzed at each of the four study visits.

Conduit Vessel Endothelial Function

Ultrasound measurement of brachial artery diameter during changes in brachial artery flow was performed as a non-invasive assessment of conduit vessel endothelial function. This technique used a 7.5 MHz linear array transducer ultrasound system (Sonos 2000, Hewlett-Packard, MA). Subjects were asked to lie still with one arm extended. A 5 cm length of the brachial artery was imaged in a longitudinal section proximal from the antecubital fossa and the probe site on the skin marked. Baseline images of the brachial artery diameter and Doppler velocities from the center of the vessel were recorded on videotape. While the images were continuously recorded, an occluding forearm cuff was inflated to 50 mm Hg above the systolic pressure for 5 minutes to exclude the hand from the studied vascular bed. Brachial artery diameter and Doppler velocities were continuously recorded before, during, and after upper arm cuff deflation. After 10 minutes, once basal diameter and flow had been restored,

nitroglycerin (400 µg) was administered sublingually and the recordings continued for 6 minutes. The ultrasound images were digitized and then analyzed using computer software. The diameter of the brachial artery was measured at a fixed distance from an anatomical marker using electronic calipers. Measurements were performed at end-diastole, coincident with the R wave on the simultaneously recorded ECG. Arterial blood flow was calculated from the Doppler flow velocity and the vessel diameter. FMD was determined as the percent change in brachial artery diameter from baseline in response to increased flow-induced shear stress. This test of conduit vessel endothelial function was performed at each of the four study visits.

ECG Recordings

During the experimental procedure for assessment of conduit endothelial vessel function (FMD), a 3-lead ECG was recorded on a videotape along with the FMD recording. The videotapes were digitized into RAW video files. From these RAW video files, time series of the ECGs were obtained at a sampling rate of 113 Hz using the Imager Software that is part of the Hemolab software (www.haraldstauss.com/Hemolab/Hemolab.html).

Heart Rate Variability Analysis

Heart rate variability was analyzed from the 113 Hz ECG time series. First, each subject's ECG was reviewed using a Hemolab module called the Analyzer. The peak intensity of each subject's R wave was identified. In order to reduce noise in the ECG recording, a low-pass Butterworth filter was applied before a beat-by-beat heart rate time

series was derived from the ECG recordings. Prior to heart rate variability analysis, artifacts, including episodes of arrhythmia, such as extrasystoles, premature ventricular contractions, premature atrial contractions, and ECG recording dropouts, were identified and replaced by interpolated values using the Analyzer software. Seven minute recordings of the ECG were utilized for the current study. Once the ECG was free of artifacts, heart rate variability analysis was performed using the Batch Processor module of Hemolab. Standard deviation of normal to normal intervals (SDNN) and the square root of the mean squared differences of successive NN intervals (RMSSD) were calculated as time-domain heart rate variability parameters.

In addition to time domain analysis, frequency domain analysis was performed in order to discriminate between sympathetic and parasympathetic contributions to heart rate variability. First, the beat-by-beat heart rate time series was converted to equidistant time series using the Analyzer module of Hemolab. The equidistant ECG time series was then imported into the Batch Processor, where spectral analysis was performed using the Fast Fourier Technique (FFT). After the new files for the power spectra were generated, the Batch Processor was again utilized to analyze these spectra and calculate the areas under the curve for the very low frequency (VLF), low frequency (LF), and high frequency (HF) bands. The utilized frequency ranges for VLF, LF, and HF can be seen in Table A1, as delineated by the Task Force of the European Society of Cardiology (43). For the purposes of this study, LF_a (absolute), LF_r (relative), HF_a, HF_r, and LF/HF (Ratio LF [ms^2]/HF[ms^2]) were calculated. Sympathetic and parasympathetic modulation of cardiac function were assessed in the low frequency range (0.04-0.15 Hz), while

parasympathetic modulation was assessed in the high frequency range (0.15-0.4 Hz) (33,47,48).

Statistical Analysis

Repeated-measures analysis of variance (ANOVA) was used to test for differences between time and frequency domain HRV parameters (SDNN, RMSSD, LF_a, LF_r, HF_a, HF_r and LF/HF (Ratio LF [ms^2]/HF[ms^2])) in the subject population between study visits #1, #2, #3, and #4. Statistical analysis was performed using the WinStat module of Hemolab (www.haraldstauss.com/Hemolab/Hemolab.html). FMD (in Δ% diameter) was also compared using repeated-measures ANOVA but only for study visit #1, #2, and #3. A p-value of <0.05 was considered to indicate statistical significance. Correlations between HRV, FMD, subject cardiovascular data, subject history, coronary artery calcification scores, and laboratory data were also computed using the R statistical software (CRAN). Laboratory data included the following measurements: ADMA, SDMA, C-reactive protein (CRP), Interleukin-6 (IL-6), creatinine, blood urea nitrogen (BUN), and glomerular filtration rate (GFR).

CHAPTER III

RESULTS

Baseline Patient Characteristics

Baseline subject characteristics are displayed in Table A2. The average subject age was 42±4 years, 75% were male, 82.02% white, 10.38% African American, and 6.6% other. A majority of patients was hypertensive (96.94%), 30.77% were diabetic, and 25.63% had a history of coronary artery disease. The etiology of renal failure in the ESRD patients was diabetes (30.7%), IgA nephropathy (18.4%), glomerulonephritis (13.2%), polycystic kidney disease (10.5%), glomerulosclerosis (7.9%), hypertension (7.9%), membranous/reflux nephropathy (7.9%), and unknown/other (3.3%)

Heart Rate Variability

Time domain and frequency domain measures of heart rate variability (HRV) were computed for the RR intervals of all subjects. Two time domain and five frequency domain HRV parameters were examined. Time domain parameters included: standard deviation of the NN intervals (SDNN) and the square root of the mean squared differences of successive NN intervals (RMSSD). Frequency domain parameters included: LF_a (absolute), LF_r (relative), HF_a, HF_r, and LF/HF (Ratio LF [ms^2]/HF[ms^2]). There were 38 subjects with complete study visits #1-3. From those, 10 subjects had also completed study visit #4. The average values of SDNN and RMSSD are shown in tables A3 and A4 and graphs C1 and C2. The average values of LF_a, LF_r, HF_a, HF_r, and LF/HF are displayed in tables A5 and A6 and graphs C3, C4, C5, C6, C7, and C8, respectively. Time domain parameters were largely maintained (did not deteriorate) up to three years

following RTX, as indicated by unchanged SDNN (p= 0.9263) and RMSSD (p=0.8207) between study visits #1-4 (N=10). Furthermore, time domain HRV also appeared to be maintained when examined solely in the large cohort of patients followed up for one year following RTX, as indicated again by relatively unchanged SDNN (p=0.6553) and RMSSD (p=0.5407) values between study visits #1-3 (N=28).

Similarly, frequency domain HRV was also largely conserved following RTX, as demonstrated in the relatively unchanged LF_r (p=0.55), LF_a (p=0.18), HF_r (p=0.40), and HF_a (p=0.27) HRV up to three years following RT (N=10). Again, when examined up to one year following RTX, LF_r (p=0.81), LF_a (p=0.39), HF_r (p=0.68), and HF_a (p=0.23) were largely maintained following transplantation (N=28). As pointed out by Chandra et al. (107) (Figure B2), a reduced LF/HF ratio of HRV (<2.5) is associated with a greater probability of cardiovascular disease events. As seen in tables A5 and A6 and graph C7 and C8, before renal transplantation, the LF/HF ratio was close to the threshold for elevated cardiovascular risk of 2.5. Three months following renal transplantation, the LF/HF ratio tended to increase above this threshold. Both one year and three years following transplantation, LF/HF decreased slightly but remained above the threshold of 2.5. These findings indicate a reduced cardiovascular risk immediately following and for up to three years after transplantation. Of particular note, an inverse correlation was observed between SDNN before renal transplantation and the reduction in plasma creatinine 3 years following renal transplantation (N=10, R= -0.60378, p=0.06455) (Graph C9), indicating the predictive value of time domain HRV for renal function up to 3 years after RTX.

As can be seen in Graph C10, a positive correlation was discovered between RMSDD 3-years after RTX and glomerular filtration rate (GFR) 1-year post RTX (R= 0.558537, p=0.11803, N=9). Additionally, an inverse correlation was also observed between blood urea nitrogen (BUN) 1-year post RTX and both RMSSD 3-years post RTX (R= -0.62384, p=0.07258, n=9) (Graph C11) and SDNN 3-years post RTX (R= -0.75444, p=0.05667, n=9) (Graph C12). Similarly, an inverse correlation was also observed between plasma creatinine 1-year post RTX and RMSSD 3-years post RTX (r= -0.61194, p=0.079874, n=9) (Graph C13). These correlations between renal function parameters and HRV suggest that deteriorating renal function contributes to loss of HRV later on.

As demonstrated in Graph C14, an inverse correlation also was discovered between symmetric dimethylarginine (SDMA), an endogenously produced inhibitor of nitric oxide synthase, 3-years post RTX and GFR 1-year post RTX (r= -0.70926 p=0.11450, n=6). Additionally, a positive correlation was observed between SDMA 3-years post RTX and BUN 1-year post RTX (r=0.785452, p=0.06410, n=6) (Graph C15). Lastly, a positive correlation was also observed between SDMA 3-years post RTX and plasma creatinine 1- year post RTX (r=0.755581, p=0.082309, n=6) (Graph C16). Together, these correlations indicate that deteriorating renal function at 1 year after RTX leads to endothelial dysfunction 3 years following RTX through SDMA-mediated inhibition of eNOS. Of particular note, a positive correlation was also observed between SDMA and IL-6, an inflammatory marker, at 3-years post RTX (r=0.88679, p=0.0526, n=6) (Graph C17).

Flow-Mediated Dilation

FMD was examined for each subject during study visits #1-4. However, at the time of this analysis, FMD scores only had been measured and calculated by the ultrasonographers for study visits #1-3. The values for mean percent change of blood vessel diameter for study visits #1-3 are reported in table A7 and graph C18. Percent change of blood vessel diameter was calculated using equation 1:

$$\text{Percent change} = \left\{\frac{[(60\ seconds\ post\ deflation)-(Base)]}{(Base)}\right\}(100\%)$$

Equation 1

The mean percent change in artery diameter for study visits #1, 2, and 3 were 5.01±1.59%, 5.86±1.57%, and 8.52±2.11%, respectively. A small percent change in artery diameter is indicative for endothelial dysfunction. The calculated P value for these differences was 0.0201 (N=30), demonstrating a significant difference between brachial artery FMD between study visits #1-3. There was a significant increase found between pre-transplant FMD values and 1-year post transplant FMD values (p=0.0058), as well as a significant increase in FMD values from 3-months post-transplant to 1-year post-transplantation (p=0.0259). However, no significant differences were found between pre-transplant FMD values and 3-months post-transplant FMD values. These data demonstrate a significant improvement in endothelial vascular function up to three years following RTX. Importantly, SDNN was also found to correlate positively with FMD (R=0.4, p<0.05, N=30) (Graph C19), indicating that an improvement in endothelial vascular function may be reflected in an increase in HRV.

CHAPTER IV

DISCUSSION

Cardiac Autonomic Function

Reduced HRV has been firmly established as a significant independent risk factor for higher mortality and cardiac death in patients with cardiovascular disease and healthy populations (108,109). Studies of HRV in ESRD patients have further shown a decrement of HRV predicative of survival (110-112). In the present study, we investigated cardiac autonomic control in patients with ESRD before and 3 months, 12 months, and 36 months following RTX. Cardiac autonomic function was assessed via time-and frequency-domain HRV analysis. Interestingly, scarce literature exists on the longitudinal effects of renal transplantation on HRV in ESRD patients, focusing instead on short-term effects immediately post-transplant (113-115). Furthermore, many studies regarding HRV in ESRD patients have focused on autonomic function during hemodialysis sessions, with conflicting results (116-118). It has been well established that autonomic dysfunction is common in patients with ESRD (119, 120), and previous studies have shown that both time and frequency domain HRV parameters are substantially decreased in ESRD patients when compared with healthy controls (110,121). Furthermore, cardiac autonomic dysfunction is also widely prevalent in diabetes patients, with numerous studies showing a lack of difference in HRV parameters between ESRD patients without a history of diabetes compared to diabetics without ESRD. These data suggest that chronic kidney disease and diabetes have an equivalent detrimental effect on cardiac autonomic activity (122).

In the present study, we sought to determine if cardiac autonomic activity

(measured via HRV parameters) would improve after normalization of renal function by transplantation. In a sample size of 10 subjects examined up to 3 years post-transplant and 28 subjects examined up to 1 year post-transplant, HRV was largely maintained, with no significant differences found between time or frequency domain parameters. This finding is consistent with previous studies that have focused on the alterations in autonomic activity after RTX, reporting variable improvement of HRV parameters both 1 and 3 months after transplantation (123,124). Interestingly, in a recent study by Rubinger et al. (113), it was found that both time and frequency domain parameters increased significantly, nearly to levels identical to age-matched healthy controls, 3 months after RTX. In this particular study, however, subjects with a history of diabetes were excluded, which suggests that cardiac autonomic activity is significantly and more severely impaired in diabetic ESRD patients as compared to non-diabetic ESRD patients, perhaps due to the co-existence of uremia and diabetic neuropathy (113). This hypothesis is further supported by the fact that no significant improvement of HRV parameters were observed in the current study, in which approximately one-third (30.7%) of the subjects were diabetic. Although the exact pathological role of diabetes in CKD remains unclear, it has been well established that CKD patients with diabetes suffer significantly more cardiovascular events and greater mortality when compared to CKD patients without diabetes (122, 125).

Of particular note, the LF/HF ratio increased significantly from 2.72±0.68 pre-transplant to 3.95±0.82 three months following RTX (N=10) and from 2.60±0.57 pre-transplant to 3.16±0.78 three months following RTX (N=28). According to Chandra et al. (107), a reduced LF/HF ratio of HRV (<2.5) is associated with a greater probability of

cardiovascular disease events (Figure 2B). Both one year and three years following transplantation, LF/HF decreased slightly compared to 3 month values but remained above the threshold of 2.5, thus suggesting that renal transplantation reduces the risk of cardiovascular disease up to 3 years following transplantation due to improved cardiac autonomic control. However, despite correction of several uremic parameters by renal replacement therapies, the mortality and morbidity rates due to CVD remain higher in transplant patients as compared to age-matched controls, due primarily to the comorbid existence of both traditional CVD risk factors and post-transplant risks such as acute rejection or diabetes mellitus (113). In addition, we also observed that high SDNN before RTX indicates a greater reduction in creatinine at 3 years post-transplant, as indicated by a significant negative correlation. According to previous studies, a greater reduction in creatinine after RTX has been associated with a reduced incidence of adverse cardiovascular events (122). In fact, in a recent study by Oikawa et al. (126), assessing the prognostic value of HRV in chronic hemodialysis and transplant patients, SDNN showed the strongest relation to all-cause and cardiovascular death. In the same study, the incidence of cardiovascular death was much greater in patients with low SDNN (<75 ms), which correlated significantly with a smaller reduction in creatinine levels post-transplant (126). Thus, as can be seen, the topic of heart rate variability and its role in assessing cardiovascular outcomes in RTX patients has yet to be fully examined and appreciated. This study attempted to explore this relationship by investigating the autonomic changes that occur with transplantation and its role in prediction of cardiovascular outcomes up to 3 years following transplantation.

Endothelial Function

CVD is the most common cause of death in ESRD patients, both in patients on hemodialysis and renal transplant patients. Endothelial dysfunction, which is characterized by a reduced synthesis or bioavailability of NO, is a primary event in the development of atherosclerosis (127). Atherosclerosis, along with other comorbid CVD risk factors such as hypertension and hyperlipidemia, serves as important precursors of adverse cardiovascular events. Endothelial dysfunction has also been established as a well-known complication of chronic uremia, despite the fact that the etiology of this condition is likely multi-factorial in nature and remains to be fully understood (128). Previous studies have shown that endothelial dysfunction is highly prevalent in a large spectrum of ESRD patients, especially pre-dialysis patients (129), patients on chronic hemodialysis (130-132), and after RTX (133,134). Although these studies have indicated that chronic endothelial injury is present in ESRD patients post-transplant, there is a scarcity of literature addressing the longitudinal effects of renal transplantation on endothelial function. In the present study, we sought to investigate endothelial vascular function in patients with ESRD immediately before and 3 months, 12 months, and 36 months following RTX. Vascular function was assessed by brachial artery FMD. The main advantage of using brachial artery FMD measurement in assessing endothelial function is that it is a non-invasive test, can be easily repeated in the same patient, and can be used to study large numbers of patients (60). Based on a number of previous studies, brachial artery FMD has been shown to correlate with measures of coronary endothelial function, and is considered the “gold standard” for measurement of endothelial function (135).

The present study showed that endothelial dependent vasodilation, measured via FMD, was significantly improved one year following RTX when compared to pre-transplant values. Furthermore, FMD values were also significantly improved at 1-year post-transplant when compared to 3-months post-transplant FMD values. There were no significant differences found between pre-transplant FMD values and 3-months post-transplant FMD values. These findings are consistent with previous studies that have shown that renal transplantation improves vascular function in ESRD patients when compared to matched controls still on dialysis therapy (136-138). Based on these past studies, it has been hypothesized that the primary cause of improvement in endothelial function in renal transplant patients is the reduction of circulating uremic toxins in the body, perhaps the most important of which being asymmetrical dimethlyarginine (ADMA) and homocysteine (138,139). Additionally, risk factors for endothelial dysfunction secondary to uremia, including hemodynamic overload, anemia, electrolyte imbalance, increased oxidative stress, hypoalbuminemia and prothrombotic factors all tend to regress after the uremic state is ameliorated following transplantation, leading to improved vascular function (140). Previous studies, however, have typically assessed renal transplant patients at a single time point after transplantation (varies between 3-months to 1-year post transplant) (136-138). Thus, when compared to non-transplanted controls on dialysis therapy, it provides a limited view of endothelial function that fails to address the effects of time or interindividual subject variability on vascular function. In fact, in a recent study by Olflaz et al. (140), it was shown that the degree of endothelial function increases inversely with shorter post-transplantation period, confirming the hypothesis that time plays an important role in reversal of endothelial dysfunction

following RTX. Results from the present study further confirm this theory, with FMD values only proving significantly different from pre-transplant values 1 year, and not 3 months, following transplantation. According to the limited studies that have to date investigated the longitudinal effects of renal transplantation on endothelial function, it has been hypothesized that that endothelial function improves significantly with a longer post-transplant period due to two possible mechanisms. First, in the early post-transplant months, it is hypothesized the chronic uremic state present before transplant is maintained, although to a lesser extent, which impedes significant endothelial vascular improvement (134,140,141). Furthermore, it has also been speculated that the time elapsed between the transplantation and the endothelial function measurement is too short for a full recovery of the vascular endothelium if the measurement is taken too soon after the transplantation process. According to current knowledge, it is not yet known how much time should elapse for a full recovery of the endothelium after any surgical procedure (135). Second, it is possible that the high dosages and troughs of immunosuppressive drugs (particularly calcineurin inhibitors) administered in the early post-transplant period are more detrimental to endothelial function than the lower, more consistent dosages characteristic of the later post-transplant period (141,142). In a recent study by Morris et al., it was shown that basal and stimulated NO production from the endothelium of forearm resistance vessels is reduced in renal transplant patients treated with high doses of cyclosporine, a calcineurin inhibitor (143). This relationship is further complicated by the fact that many immunosuppressive agents contribute to hypertension, hyperlipidemia, and metabolic syndrome, all of which may be associated with endothelial dysfunction (144).

Also, it is of particular note that despite significant improvement in vascular function post-transplant, kidney transplantation does not restore endothelium-dependent vasodilation to levels of healthy controls, which is consistent with previous studies (119, 144). Thus, it can be concluded that the post-transplant state still represents a state of substantial endothelial dysfunction, undoubtingly contributing to the increased cardiovascular risk in renal transplant patients as compared to the general population. In addition, SDNN was found to correlate positively with flow-mediated dilation ($R=0.4$, $p<0.05$), suggesting that improved vascular endothelial function following transplantation is associated with improved cardiac autonomic control and possibly reduced risk for CVD events. Although the exact mechanistic link between endothelial function and autonomic function still remains under investigation, it is hypothesized that disturbed control of autonomic function in renal failure is part of a larger spectrum of functional abnormalities related to accumulation of uremic toxins (145). In fact, in a recent study by Tamura et al. (145), it was found that one of the most important clinical determinants of HRV in dialysis patients was the duration of hemodialysis, i.e., the number of years of exposure to a uremic environment. Thus, it can be speculated that cardiac autonomic function and endothelial function are closely interrelated in the state of ESRD, even extending into the post-transplant state, due perhaps to the continued presence of damaging uremic toxins in the body.

Kidney Function and HRV Post-Transplant

As previously discussed, it has been well established that patients with ESRD have a high prevalence of cardiac autonomic dysfunction, manifested primarily by

decreased heart rate variability (106,146). Following renal transplantation and subsequent normalization of renal function, however, findings regarding improvement in autonomic dysfunction are variable, with some studies showing significant improvement and others demonstrating little to no improvement (122-124). It has been hypothesized that the degree of improvement in autonomic function post-transplant is highly related to factors secondary to renal function, such as presence of hypertension, diabetes mellitus, or other comorbid cardiovascular diseases (124). In fact, in previous studies, it has been shown that HRV, particularly HF HRV, is significantly reduced in patients with coronary artery disease and heart failure, indicating a shift to sympathetic predominance (147,148). Thus, in transplant patients with comorbid cardiovascular complications, it can be speculated that complete reversal of autonomic dysfunction is blunted by autonomic abnormalities that occur secondarily to these cardiovascular complications.

In the present study, it was demonstrated that RMSSD 3-years following renal transplant was positively correlated with GFR 1-year post transplant. GFR is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time and is commonly utilized to assess the excretory function of the kidneys. Thus, a lower calculated GFR would be indicative of poor renal function. Based on the above correlation, it can be speculated that low GFR 1-year after renal transplant predicts low HRV (RMSSD) 3-years post-transplant. Because it has been established that reduced HRV is a significant independent risk factor for higher mortality and cardiac death, it can be said that low GFR 1-year post-transplant consequently predicts poor cardiovascular outcomes (119). Few previous studies have examined the relationship between post-transplant GFR values and cardiovascular outcomes; however, it has been demonstrated

in a number of studies that GFR in ESRD patients on chronic hemodialysis correlates highly with cardiovascular events and mortality (108,109,119), thus lending support to our claim that post-transplant GFR may be utilized to predict cardiovascular events in the near future. In addition, based on the above correlation, it was not surprising to discover an inverse correlation between BUN 1-year post RTX and RMSDD values 3-years post-transplant. BUN is a measure of the amount of nitrogen in the blood in the form of urea, and, like GRF, it is a commonly used indicator of renal function (3). Urea is a by-product from metabolism of proteins by the liver and is removed from the blood by the kidneys, therefore high levels of blood urea nitrogen indicate poor renal functioning (5). Thus, it can be said that, in much the same manner as GFR, high plasma urea values can predict low HRV (RMSSD) 3 years after RTX. Along with plasma urea, a more complete estimation of renal function can be made when also analyzing the plasma concentration of creatinine. Creatinine is a breakdown product of creatinine phosphate in muscle and is predominantly filtered out of the blood by the kidneys (7). However, if the filtering of the kidneys is poor, creatinine blood levels rise. Therefore, creatinine levels in the blood can be used to calculate the creatinine clearance (CrCl), which reflects the GFR (1). Therefore, an inverse correlation was also observed between plasma creatinine 1-year post-transplant and RMSSD 3-years post-transplant, which suggests that high plasma creatinine 1 year after transplant predicts low HRV 3-years post-transplant. Taken together, these correlations indicate that poor renal function 1 year following RTX predicts low HRV and, consequently, increased cardiovascular events 3 years following RTX.

Kidney Function and Inflammation Post-Transplant

As has been discussed previously, traditional risk factors fail to sufficiently explain the high prevalence of CVD in CKD. As a result, this has recently prompted further investigation into non-classic risk factors, and, according to initial studies, inflammation and oxidative stress are thought to play the most prominent roles (149). Despite this recent progress, however, the exact source of the oxidative stress and inflammation in CKD still remains unknown, although several prime candidates are now emerging. The most widely investigated uremic toxin is asymmetric dimethylarginine (ADMA), an inhibitor of nitric oxide synthase (NOS). ADMA has been consistently demonstrated to be elevated in both predialysis and dialysis CKD patients (150-152). Today, ADMA is generally accepted as a marker of endothelial dysfunction and a strong predictor of CVD in both the general population and CKD patients (153). Until recently, little attention had been paid to symmetric dimethylarginine (SDMA), the structural isomer of ADMA, until new studies demonstrated the clinical importance of SDMA as an independent cardiovascular risk factor for CKD patients (154-157). Since this finding, the physiological link between CVD and SDMA has been widely investigated, with preliminary in vitro studies revealing that the addition of SDMA to monocytic cell lines resulted in a significant increase in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (158). NF-κB acts as a key transcription factor regulating genes encoding proinflammatory mediators such as cytokines, cell adhesion molecules, and acute-phase proteins (159). In fact, a recent study by Schepers et al. further confirmed this link by demonstrating that in vitro SDMA induced increased tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), both cytokines involved in the process of

systemic inflammation (158). Thus, these data suggest a causal contribution of SDMA to the chronic inflammatory status that characterizes the uremic condition in ESRD patients. In the present study, we confirmed the inflammatory character of SDMA by demonstrating a positive correlation between SDMA serum levels and inflammatory parameter IL-6 3 years post-transplant.

Unlike the majority of previous studies regarding SDMA in ESRD patients that have solely focused on inflammatory markers, we also examined the relationship between kidney function and SDMA. Interestingly, an inverse correlation was discovered between SDMA 3-years post RTX and GFR 1-year post RTX. This finding is supported by a recent study that also demonstrated an inverse correlation between GFR and SDMA (159). However, unlike this past study, which correlated GFR and SDMA at the same point in time, we demonstrated a longitudinal correlation between the two that may suggest the ability to predict SDMA levels and hence, inflammation levels via renal function. Consistent with this noted correlation, we also demonstrated a positive correlation between SDMA 3-years post RTX and both BUN and plasma creatinine 1-year post RTX. These correlations further lend support to the claim that renal function may be utilized as a prognostic tool for SDMA levels. Because it has been previously established in a number of studies that the presence of low-grade systemic inflammation in patients with ESRD is associated with endothelial dysfunction, we can speculate that poor renal function thus predicts endothelial dysfunction (128, 160,161). Furthermore, endothelial dysfunction is a well-known predictor for cardiovascular morbidity and mortality, thus additionally suggesting the conclusion that poor renal function can, in turn, be utilized as a tool to predict adverse cardiovascular events (128).

As we have demonstrated in the present study, there is a significant correlation between poor renal function 1 year post-transplant (established by poor GFR, high BUN, and high plasma creatinine levels) and both RMSSD and SDMA levels 3 years post-transplant. As previously stated, RMSSD is a measure of cardiac autonomic function, and SDMA can be used as an inflammatory marker in ESRD patients. However, because no causal relationships can be determined from the above correlations, it is still unknown whether poor renal function causes inflammation, which in turn leads to poor autonomic function or poor renal function leads first to autonomic dysfunction, which subsequently leads to inflammation and endothelial damage. It is likely that the two factors are closely interrelated, with the accumulation of uremic toxins that occurs with ESRD perhaps causing concomitant occurrence of both. It is also interesting to note that significant correlations were not observed between ADMA and GFR, BUN, and creatinine, despite the close structural relationship between SDMA and ADMA. This finding may be attributed to differences in removal from the body. While, in normal renal function, approximately 80% of ADMA is eliminated by the enzyme dimethylaminolase (DDAH) and 20% by renal excretion, SDMA is completely eliminated by the kidneys (162). Thus, it is not surprising that in a recent meta-analysis based on 18 studies, SDMA was suggested as a marker of renal function (163). Lastly, based on a recent clinical study, a negative correlation was demonstrated between statin use and concentration of SDMA in CKD patients (158). Thus, in further studies, it may be interesting to examine the effect of an intervention with statins on endothelial function and renal function during progression of CKD or following RTX.

CHAPTER V

CONCLUSION

In conclusion, renal transplantation in ESRD patients chronically improves endothelial vascular function for up to 1 year and temporarily improves cardiac autonomic control (LF/HF ratio). High HRV (SDNN) before renal transplantation was found to correlate with a greater reduction in plasma creatinine after renal transplantation, and this greater reduction in creatinine has been associated with a reduced incidence of adverse cardiovascular events. HRV (SDNN) was demonstrated to correlate significantly with FMD, suggesting that improved vascular endothelial function following transplantation is associated with improved cardiac autonomic control. We speculate that improved endothelial vascular function contributes to reduced cardiovascular events following renal transplantation in ESRD patients. Finally, renal function 1 year following renal transplantation predicts low HRV (RMSSD) and high SDMA 3 years after renal transplantation, which may predict adverse cardiovascular outcomes.

CHAPTER VI

LIMITATIONS

We recognize several limitations of the present study. First, the study included a relatively small number of subjects, with only a small subset of this population being examined up to three years after transplantation. A larger sample size may have allowed for a higher statistical power and detection of more subtle differences between time points after RTX. Furthermore, significant correlations that were observed were based on a limited subset of the population with data available up to three years after transplantation, thus introducing the possibility that additional correlations existed but failed to reach significance to the limited sample size. Second, because the study was relatively open in regards to subject recruitment, this resulted in a non-homogenous subject population in regards to clinical comorbidities, with nearly all subjects displaying one or more comorbidities that may have confounded the effects of RTX on both cardiac autonomic function and endothelial function.

Additionally, the technique that was utilized to measure endothelial function may have also served as a limitation in itself. Although brachial artery FMD measurement by ultrasonography is a non-invasive and easily producible technique, it is also operator-dependent. Furthermore, at the time of this analysis, FMD scores only had been measured and calculated by the ultrasonographers up to study visit #3, limiting analysis of endothelial function to up to one year following transplantation. Thus, comparison of endothelial function with kidney function and cardiac autonomic control three years post-transplant was not possible due to a lack of available data at the time of analysis.

APPENDIX A. TABLES

Table A1. Selected Frequency Domain Measures of HRV

Analysis of Short-term Proceedings (5 min)

Variable	**Units**	**Description**	**Frequency Range**
VLF	ms^2	Power in VLF range	≤0.04 Hz
LF	ms^2	Power in LF range	0.04-0.15 Hz
LF norm	nu	100xLF power/total power	
HF	ms^2	Power in HF range	0.15-0.4 Hz
HF norm	nu	100xHF power/total power	
LF/HF		Ratio LF [ms^2]/HF[ms^2]	

Source: Task Force of the European Society of Cardiology the North American Society of Pacing and Electrophysiology. 1996. Heart Rate Variability. *Circulation*. 93:1043-1065.

Table A2. Baseline Subject Characteristics

Baseline Subject Characteristics	**Overall n=38**
Current Enrollment	38 ESRD Patients
Demographics/anthropometrics	
Age	42±4 years
Gender	
Male	75%
Race	
White	82.02%
Black	10.38%
Hispanic	3.77%
Asian	1.89%
Native American	0.94%
Body mass index (kg/m^2)	36.8±5.7
Smoker	20.41%
Blood Pressure	141±4 / 79±2 mmHg (borderline HT)
Creatinine	6.6±0.8 mg/dL
Heart Rate	71±2 bpm
Comorbidities	
Hypertension	96.94%
Hyperlipidemia	62.24%
Obesity	36.11%
History of Coronary Artery Disease	26.53%
Diabetes	
Type I	8.02%
Type II	22.68%

Table A3. Time domain heart rate variability parameters for patients with all 4 visits

Heart Rate Variability Parameters	**Visit #1 n=10**	**Visit #2 n=10**	**Visit #3 n=10**	**Visit #4 n=10**	**P**
SDNN (ms)	36.04±3.84	38.62±4.65	36.92±5.90	39.85±4.11	0.93
RMSSD (ms)	20.70±1.87	23.30±5.30	25.30±5.38	21.65±2.29	0.82

Table A4. Time domain heart rate variability parameters for patients with visits #1-3

Heart Rate Variability Parameters	**Visit #1 n=28**	**Visit #2 n=28**	**Visit #3 n=28**	**P**
SDNN (ms)	43.95±3.76	41.11±3.07	43.03±3.55	0.66
RMSSD (ms)	37.70±4.24	33.56±3.64	34.97±3.99	0.54

Table A5. Frequency Domain heart rate variability parameters for patients with all 4 visits

Heart Rate Variability Parameters	**Visit #1 n=10**	**Visit #2 n=10**	**Visit #3 n=10**	**Visit #4 n=10**	**P**
LF_a (bpm^2)	2.00±0.67	2.08±0.51	1.89±0.53	1.77±0.44	0.18
LF_r (bpm^2)	30.98±4.31	32.66±3.40	29.70±3.50	30.84±3.27	0.55
HF_a (bpm^2)	0.97±0.31	0.93±0.35	1.23±0.56	0.62±0.15	0.27
HF_r (bpm^2)	17.89±3.89	13.85±3.88	15.80±4.17	12.09±1.49	0.40
LF_a / HF_a (no units)	2.72±0.68	3.95±0.82	3.49±0.94	2.86±0.44	0.19

Table A6. Frequency domain heart rate variability parameters for patients with visits #1-3

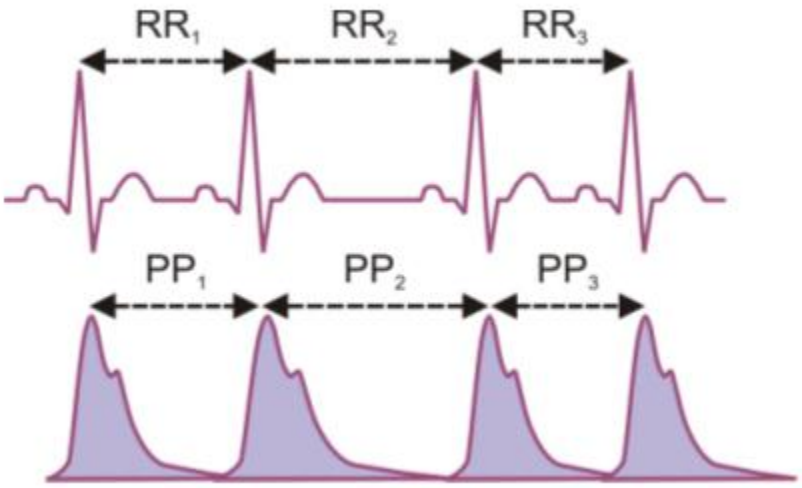
Heart Rate Variability Parameters	**Visit #1 n=28**	**Visit #2 n=28**	**Visit #3 n=28**	**P**
LF_a (bpm^2)	1.85±0.32	2.37±0.56	1.82±0.31	0.39
LF_r (bpm^2)	27.09±2.51	28.61±0.55	27.28±2.03	0.81
HF_a (bpm^2)	0.71±0.13	0.75±0.19	0.64±0.11	0.23
HF_r (bpm^2)	15.72±3.31	18.56±4.73	17.39±3.92	0.68
LF_a / HF_a (no units)	2.60±0.57	3.16±0.78	2.84±0.51	0.54

Table A7. Flow Mediated Dilation for patients with visits #1-3

	Visit #1 n=30	**Visit #2 n=30**	**Visit #3 n=30**	**P**
Change in vessel diameter (%)	5.01±1.59	5.86±1.57	8.52±2.11	0.02

APPENDIX B. FIGURES

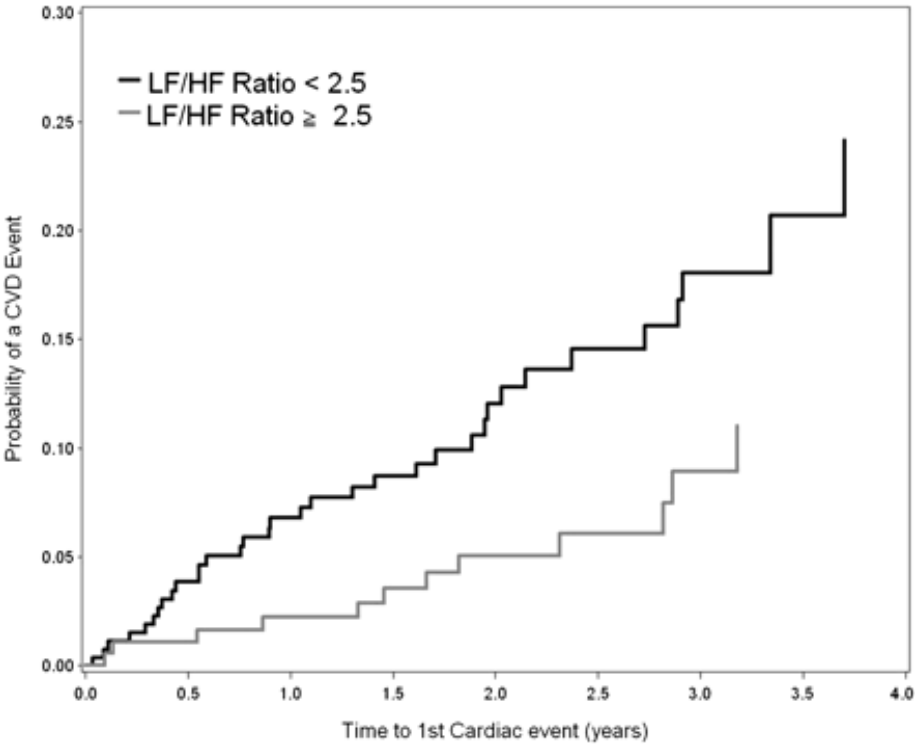
Figure B1. Heart Rate Variability



Source: BioCom Technologies. Copyright © 1996-2009

Note: Heart rate variability refers to the variations in the time intervals between "normal beats" that originate from the sinus node. HRV is measured as the R-to-R interval between 2 beats, as can be seen in the ECG trace (top). Alternatively, heartbeats can be detected using a pulse wave signal derived from a photoplethysmograph (PPG), as depicted in the PPG signal (bottom). HRV is then calculated based on these P-P intervals.

Figure B2. LF/HF Ratio

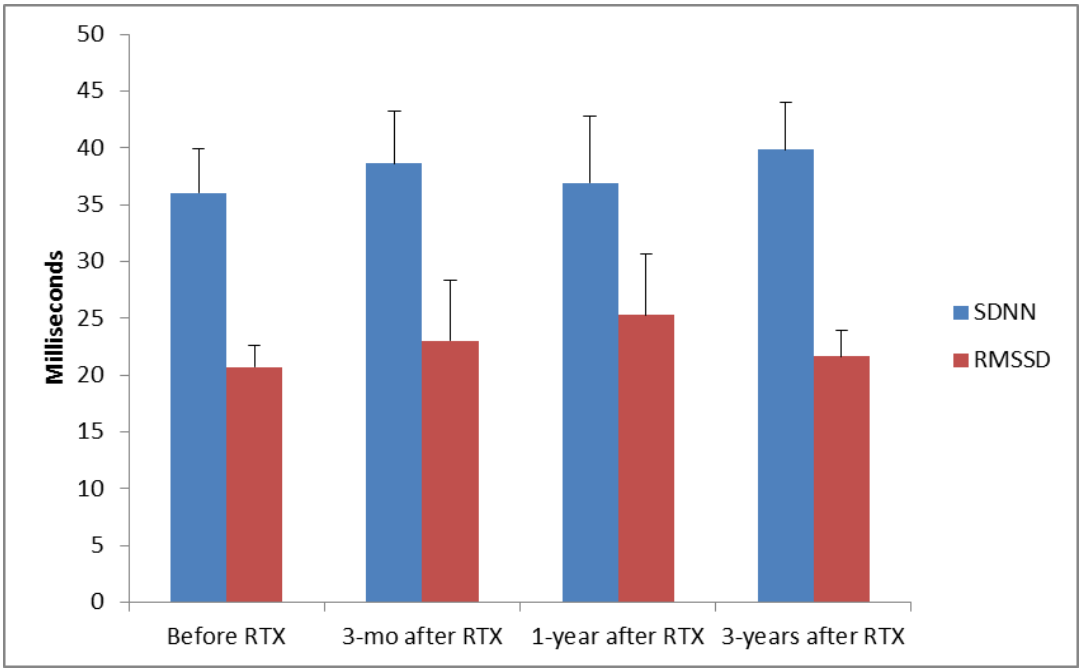


Source: Chandra P, Sands RL, Gillespie BW, Levin NW, Kotanko P, Kiser M, Finkelstein F, Hinderliter A, Pop-Busui R, Rajagopalan S, Saran R. 2012. Predictors of heart rate variability and its prognostic significance in chronic kidney disease. *Nephrol. Dial. Transplant* 27.2:700-709.

Note: Chandra et al. reported that reduced LF/HF ratio of HRV (<2.5) is associated with a greater probability of cardiovascular disease events.

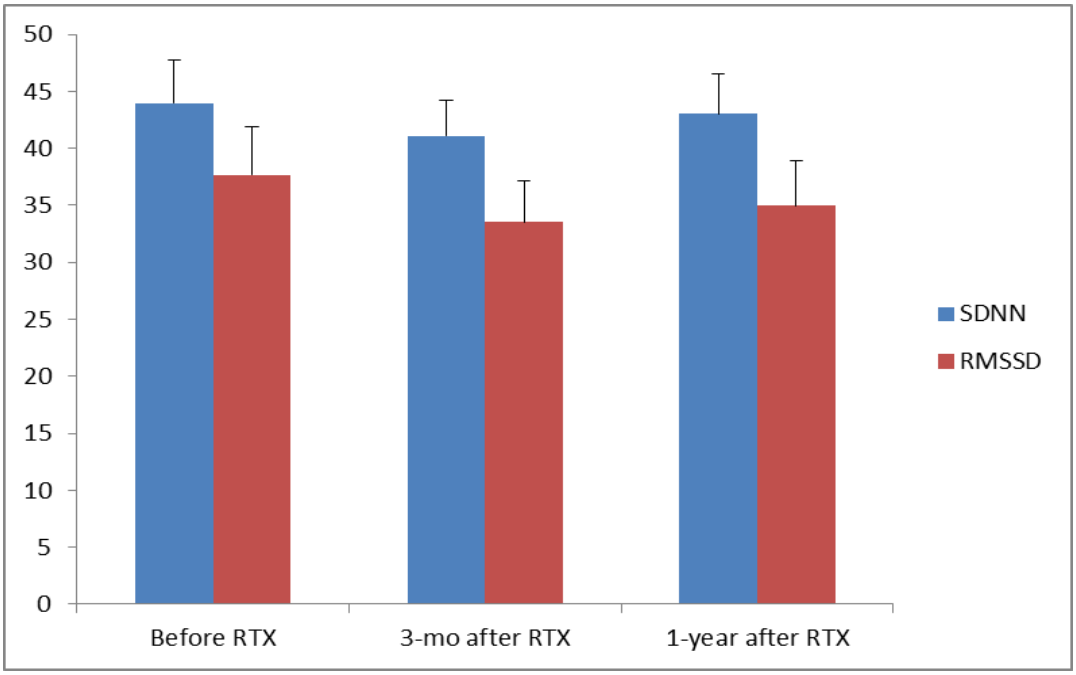
APPENDIX C. GRAPHS

Graph C1. Time domain heart rate variability for patients with all 4 visits



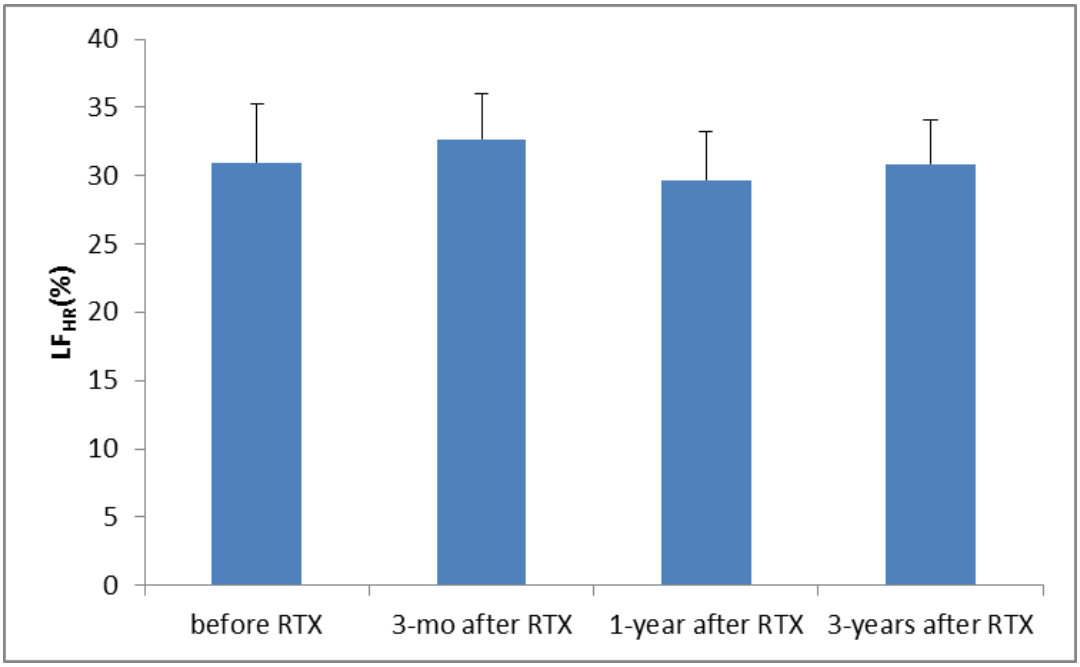
Note: SDNN (p= 0.9263), RMSSD (p=0.8207), N=10

Graph C2. Time domain heart rate variability for patients with visits #1-3



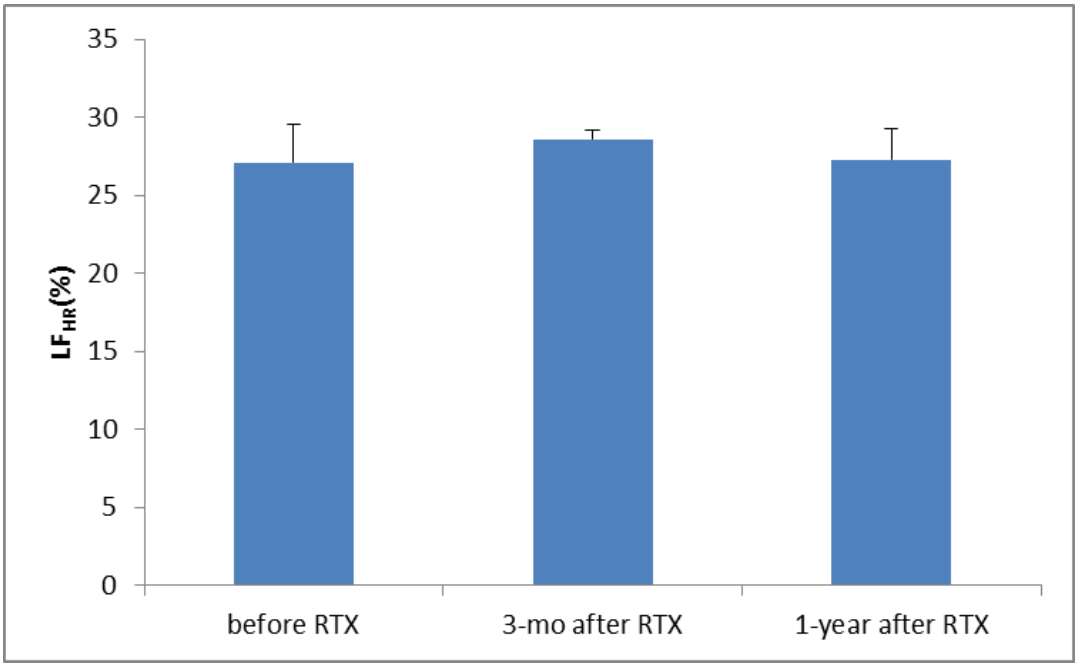
Note: SDNN (p=0.6553), RMSSD (p=0.5407), N=28

Graph C3. Frequency domain LF_r (%) for patients with all 4 visits



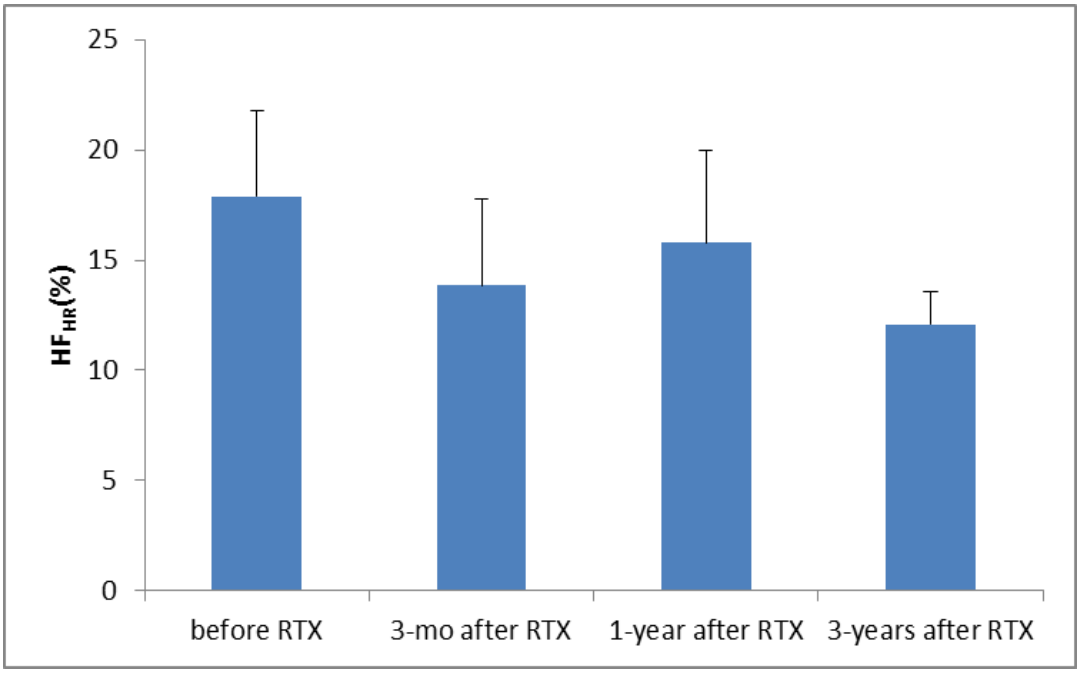
Note : LF_r (p=0.5526), N=10

Graph C4. Frequency domain LF_r (%) for patients with visits #1-3



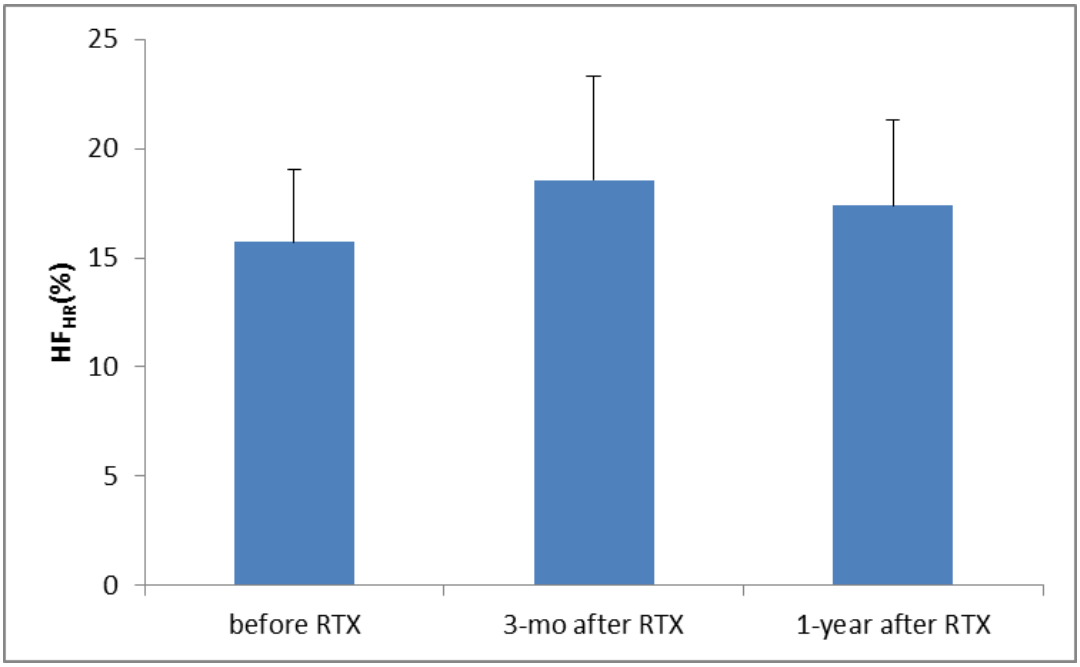
Note: LF_r (p=0.8105), N=28

Graph C5. Frequency domain HF_r (%) for patients with all 4 visits



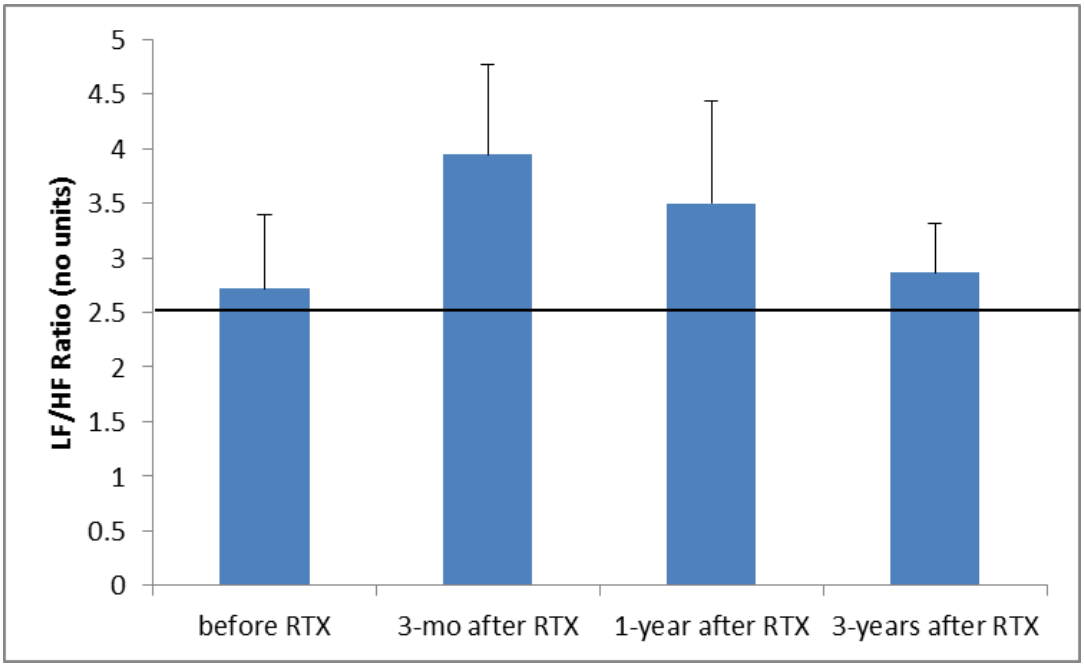
Note: HF_r (p=0.4011), N=10

Graph C6. Frequency domain HF_r (%) for patients with visits #1-3



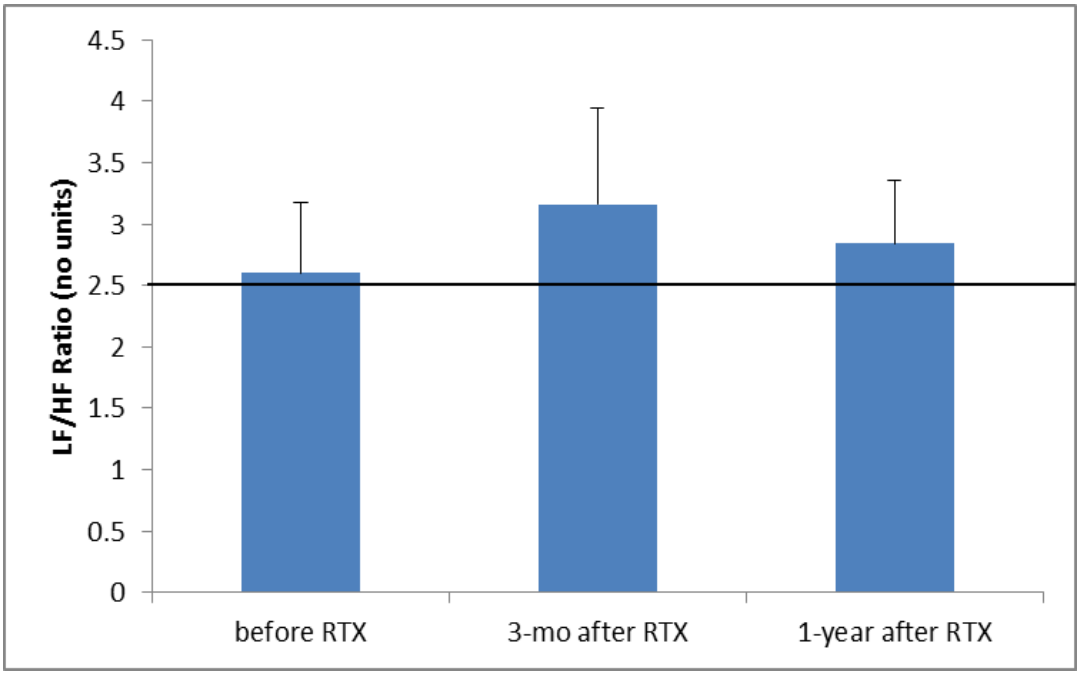
Note: HF_r (p=0.68), N=28

Graph C7. Frequency Domain LF_a / HF_a for patients with all 4 visits



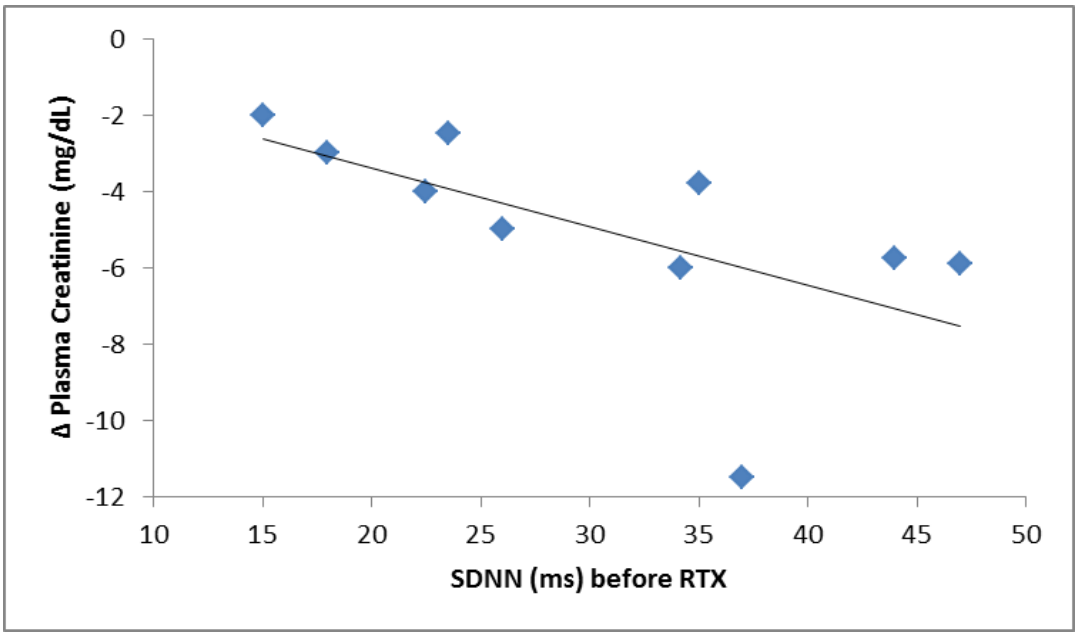
Note: LF_a / HF_a (p=0.1874), N=10. Horizontal line represents threshold for elevated CVD risk. Values below this line indicate elevated CVD risk according to Chandra et al. (107).

Graph C8. Frequency domain LF_a / HF_a for patients with visits #1-3



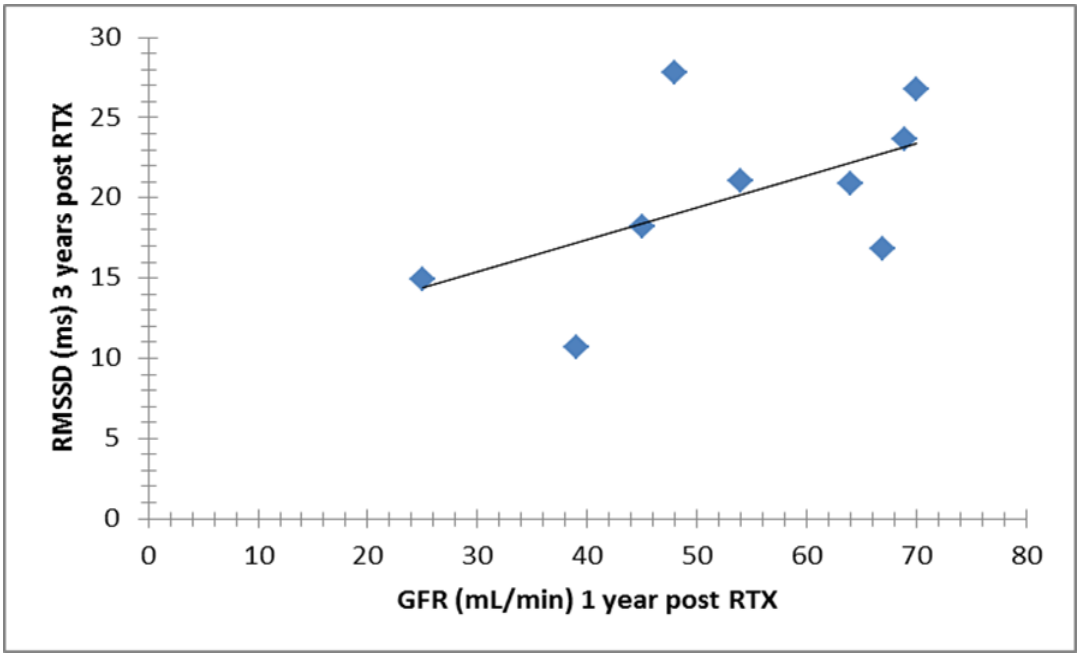
Note: LF_a / HF_a (p=0.5427), N=28. Horizontal line represents threshold for elevated CVD risk. Values below this line indicate elevated CVD risk according to Chandra et al. (107).

Graph C9. SDNN before RTX vs. Δ in Plasma Creatinine from before RTX to 3 years after RTX



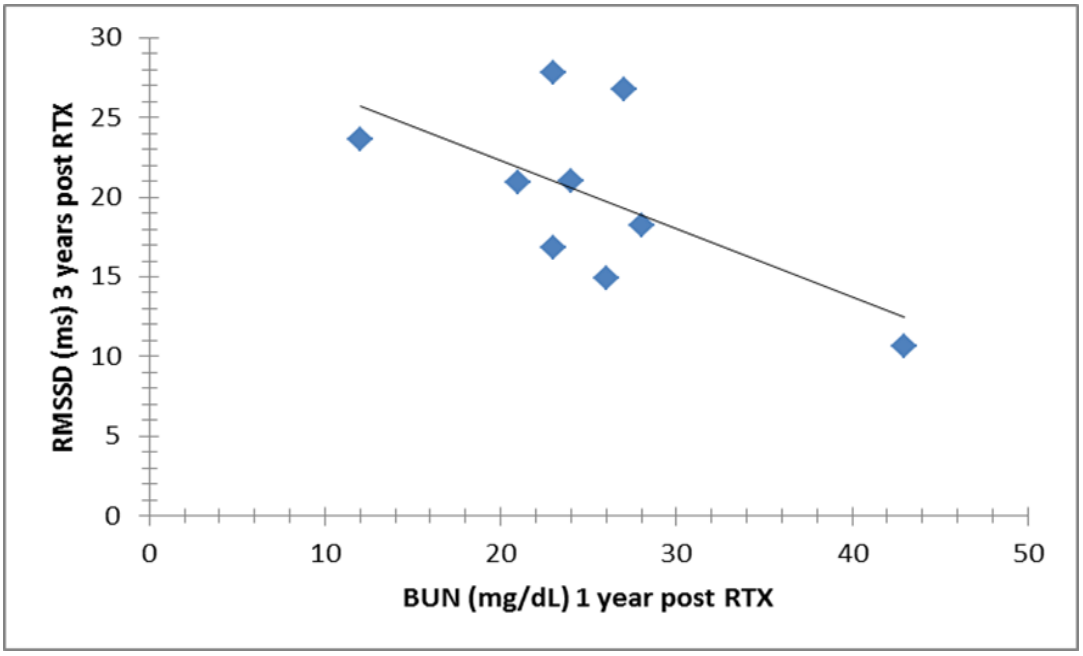
Note: r= -0.60378, p=0.06455, n=10

Graph C10. RMSSD 3 years post RTX vs. GFR 1 year post RTX



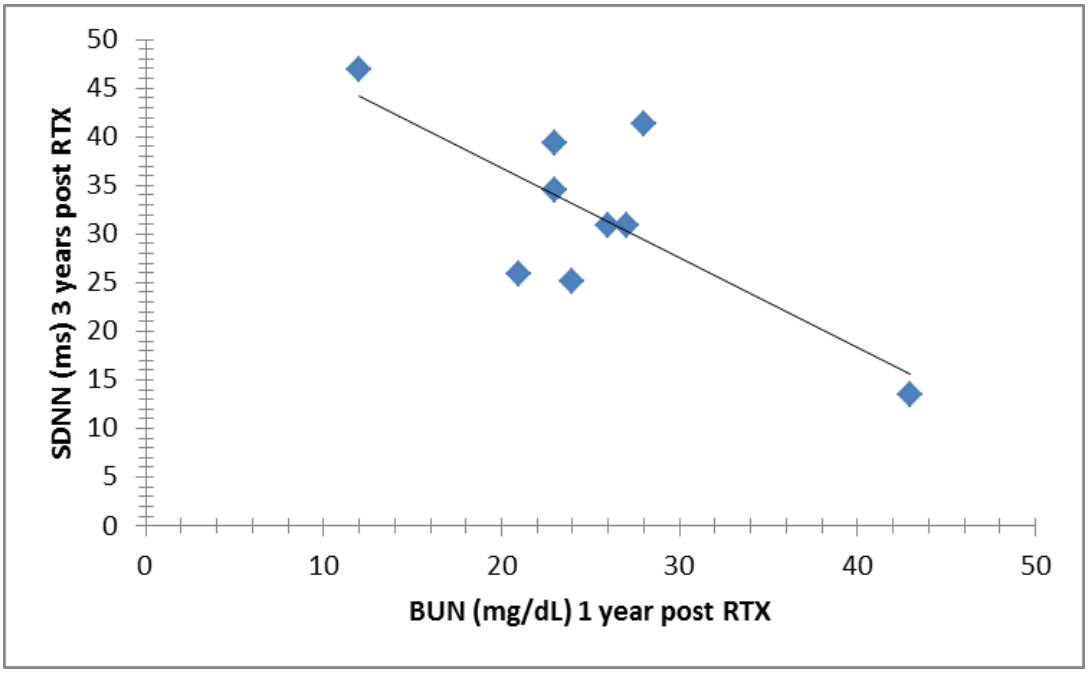
Note: r= 0.558537, p=0.11803, n=9

Graph C11. RMSSD 3 years post RTX vs. BUN 1 year post RTX



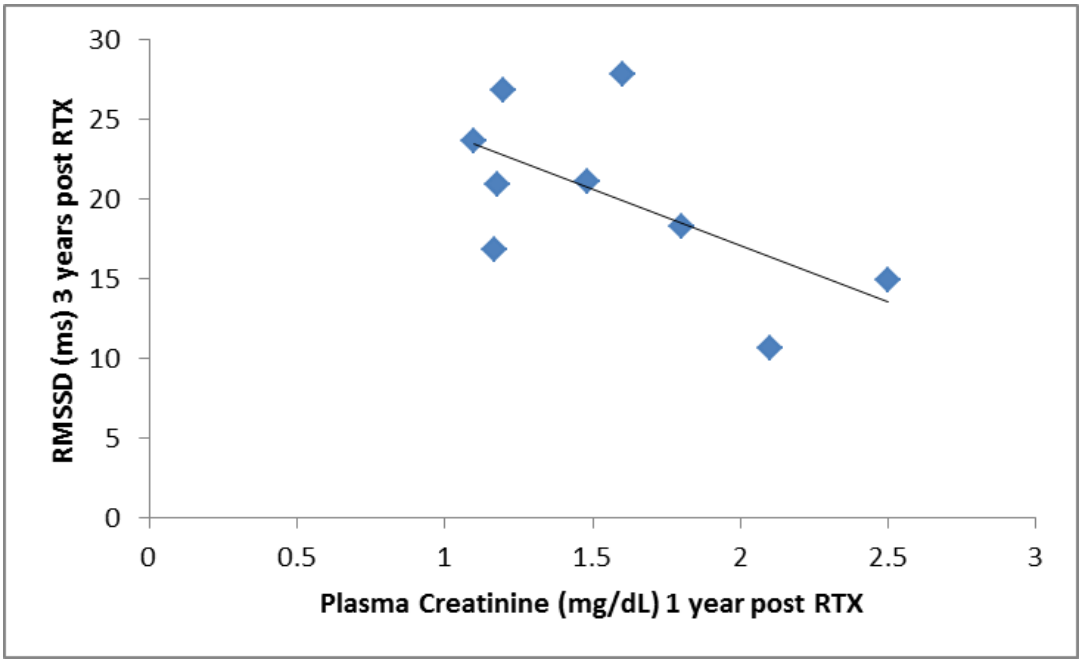
Note: r= -0.62384, p=0.07258, n=9

Graph C12. SDNN 3 years post RTX vs. BUN 1 year post RTX



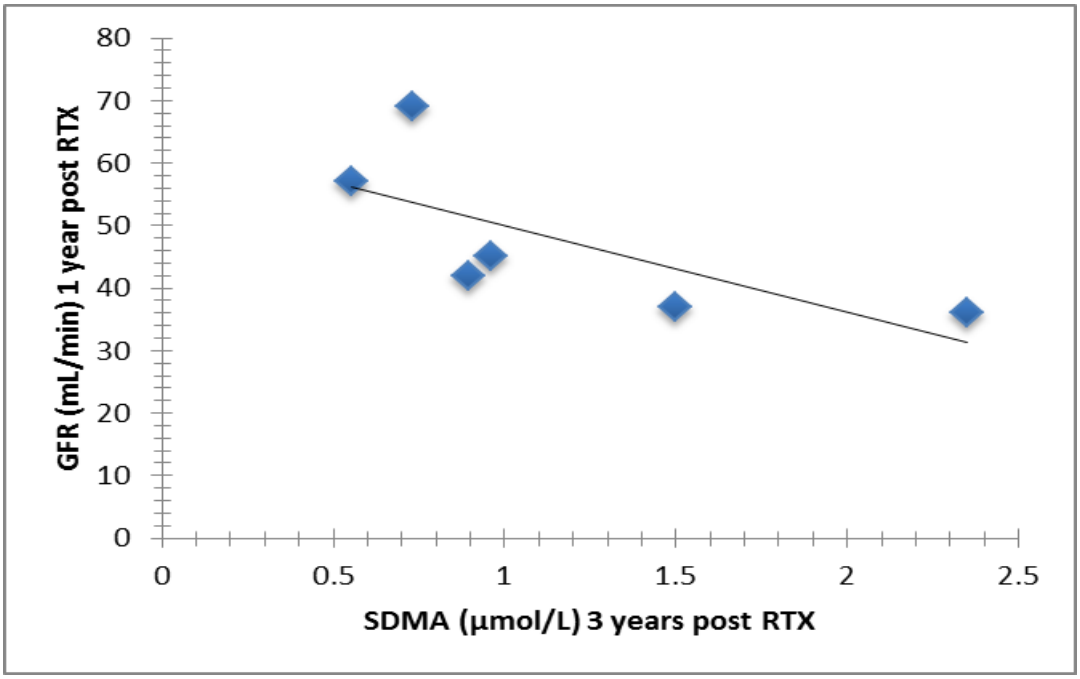
Note: r= -0.75444, p=0.05667, n=9

Graph C13. RMSSD 3 years post RTX vs. Plasma Creatinine 1 year post RTX



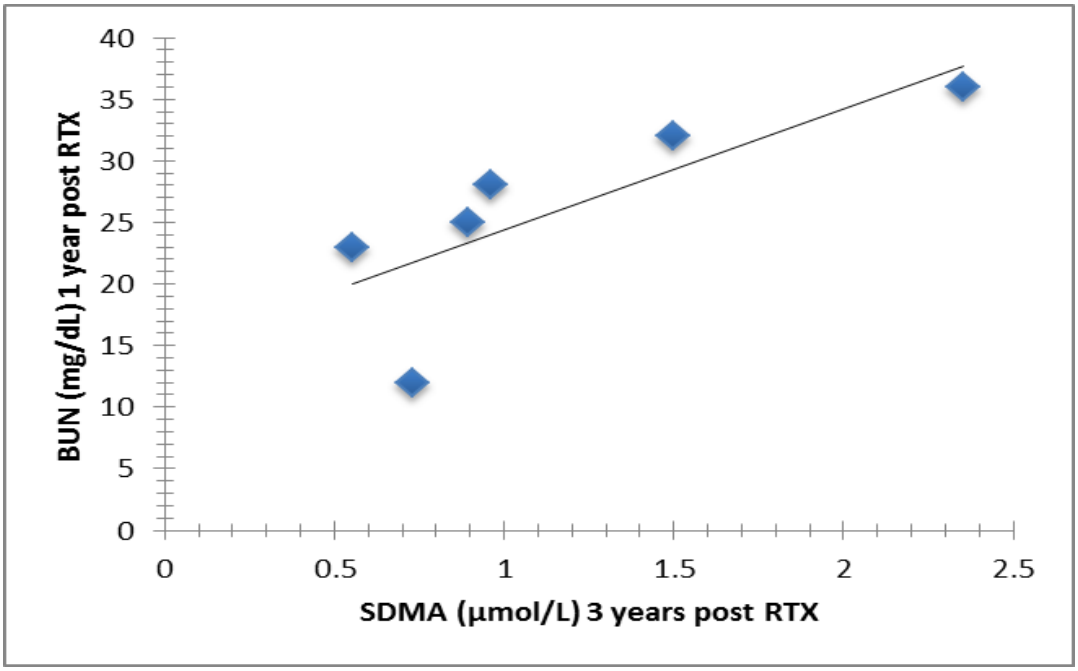
Note: r= -0.61194, p=0.079874, n=9

Graph C14. SDMA 3 years post RTX vs. GFR 1 year post RTX



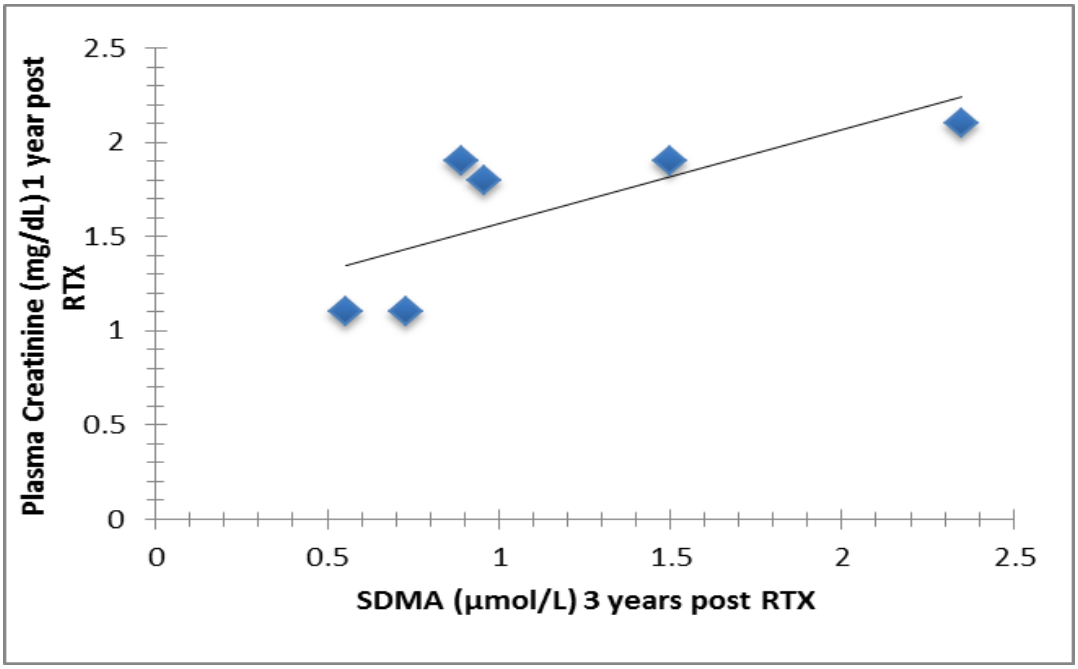
Note: r= -0.70926 p=0.11450, n=6

Graph C15. SDMA 3 years post RTX vs. BUN 1 year post RTX



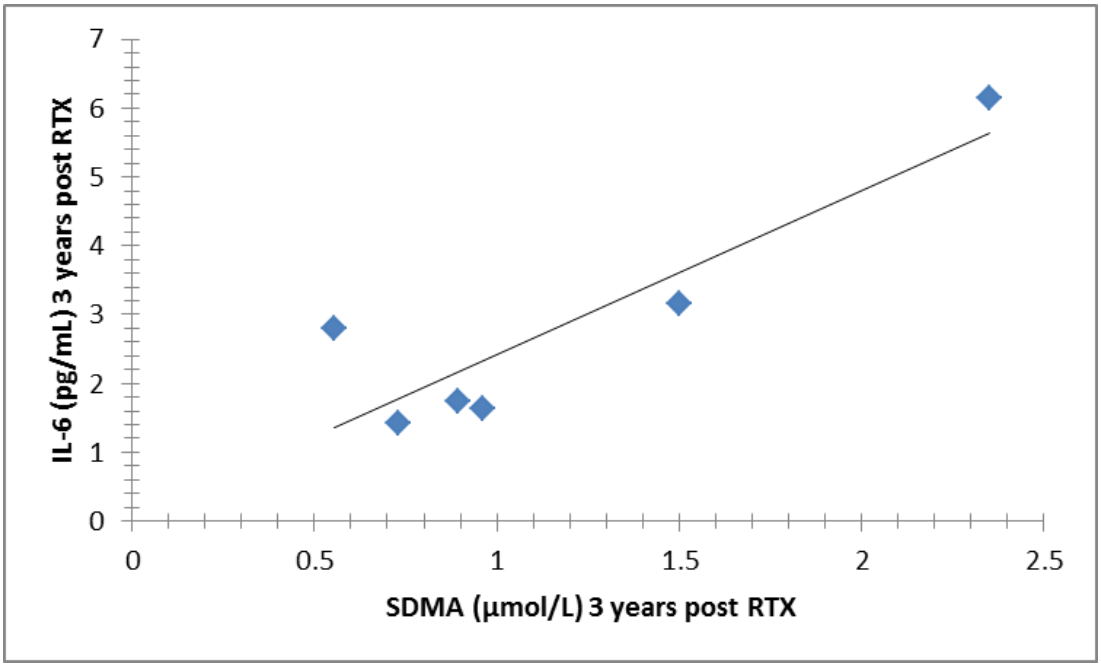
Note: r= 0.785452, p= 0.06410, n=6

Graph C16. SDMA 3 years post RTX vs. Plasma Creatinine 1 year RTX



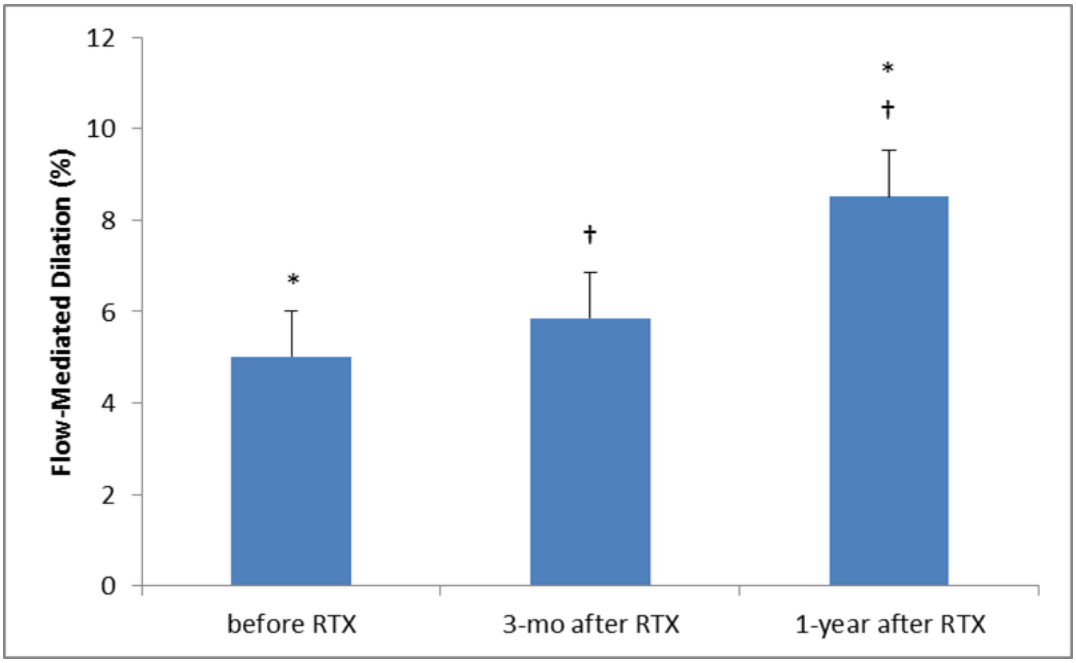
Note: r= 0.755581, p= 0.082309, n=6

Graph C17. IL-6 3 years post RTX vs. SDMA 3 years post RTX



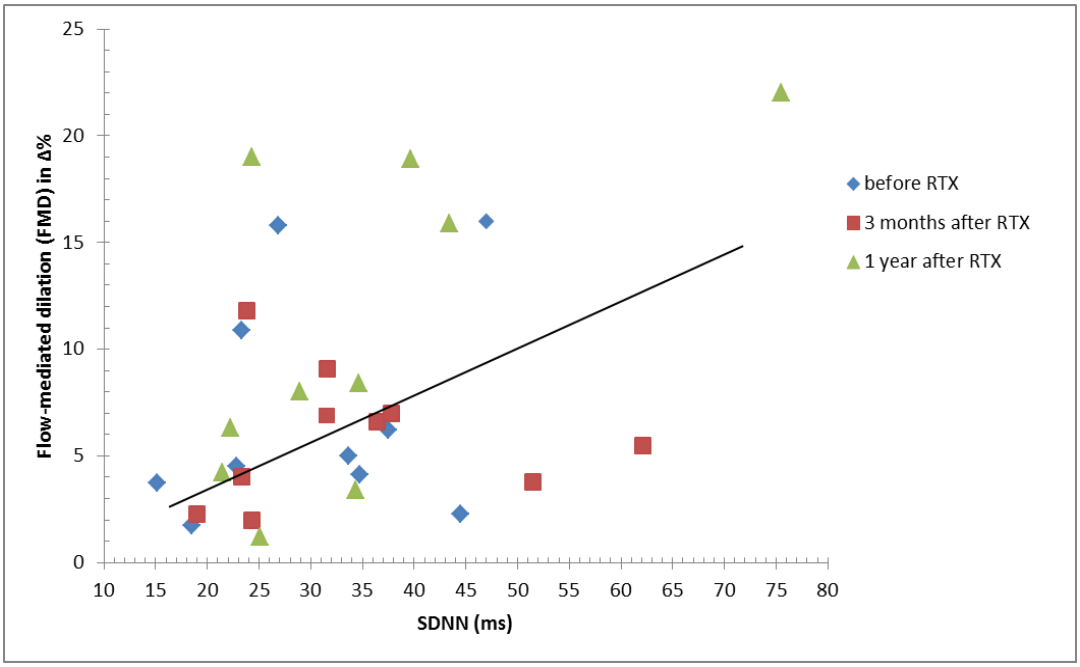
Note: r=0.88679, p=0.0526, n=6

Graph C18. Flow-mediated dilation for study visits #1-3



Note: FMD (p=0.0201), N=30
*p=0.0058
†p=0.0259

Graph C19. SDNN vs. Flow-mediated dilation in Δ%



Note: r=0.4, p<0.05, n=30

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