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Periodontitis, Inflammatory Markers and Solid Organ Transplant Recipients

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Periodontitis, Inflammatory Markers and Solid Organ Transplant Recipients

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Dedication

I dedicate this work to my family. My wife, Muzna, whose love, care and support made it possible for me to overcome hardships and pursue my goals. My children, Yanal and Noor, for they bring endless joy and happiness to my every day. My mother, Ilham, for she has lovingly nurtured me into the man I am today. My sister, Nourhan, and my brothers, Emad & Hamza, for the limitless love, support and encouragement they provide me.

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I. General Introduction

A. Renal and Cardiac Transplantation: overview and current challenges

The first successful solid-organ transplantation procedure was performed in Boston, USA, in 1954. A kidney was transplanted from a healthy donor to his identical twin suffering from kidney failure.[1] Although attempts at organ transplantation had been carried out decades prior to that, they were unsuccessful.[2] Since this first-reported successful transplantation,[1] the field of organ transplantation has witnessed tremendous advancements in scope, number of procedures and outcome quality. Today, organ transplantation is considered the treatment of choice for end-stage organ failure. In 2002, 150,000 individuals were living in the US with functioning allografts compared to 62,000 in 1993.[3]

Despite the great strides that were made in the field of organ transplantation, several challenges continue to face clinicians, researchers and health care planners alike, as well as -very importantly- patients. The 1-year renal and cardiac transplant survival (90-95% and 87%, respectively) has improved since the dawn of transplantation. Nevertheless, the 10-year survival rates fall dramatically to 51-68% and 50%, respectively, with modest improvements over the past two decades.[4-6] This is In spite of lower rates of acute rejection episodes.[7] Progressive allograft tissue damage and deterioration of graft function is observed in all transplanted organs and is collectively and generically called “chronic allograft dysfunction”. Intervention to effectively prevent or interrupt this process remains a challenge.[8]

Another challenge is that transplant patients are placed on long-term immunosuppression. Clearly, Immunosuppressive drug regimens reduced the rate of

acute rejection,[8] but they have side-effects which could injure the allograft itself[9] or cause an increased susceptibility to infections or malignancy.[10] The ultimate goal of immunosuppression or "immuno-intervention" is to achieve immunologic tolerance; intervening at an early stage of transplantation, through manipulation of the immune response, so that the recipient immune system no longer identifies the allograft as foreign.[11] However, trials that tested the impact of withdrawal of immunosuppressant medication showed deterioration of graft function for the majority of the patients.[12, 13]

A multitude of medical conditions and co-morbidities complicate the medical care of transplant recipients and pose another major challenge. One study reported that approximately 30% of all deaths in renal transplant patients after the first year are due to cardiovascular complications.[14] Infection and malignancies account for 11% and 10%, respectively, of such deaths.[14] Diabetes is highly prevalent in transplant patients (20%)[15] and constitutes in itself another risk factor for cardiovascular diseases. Additionally, immunosuppressive therapy using calcineurin inhibitors and corticosteroids has been associated with higher incidence of diabetes after transplantation. [16]

More than 50 years have passed since the first report of a successful solid-organ transplantation procedure. Tremendous advancements have taken place in all different aspects of the transplantation process, which in turn made this treatment option a widely applied therapy for end-stage organ failure. A host of challenges, however, need to be overcome to optimize outcomes and reduce risks of this therapeutic modality. The continued research in the field of organ transplantation and cellular and molecular biology will hopefully bring us closer to that goal.

B. Chronic Allograft Nephropathy (CAN): etiology and mechanisms

Although the term CAN has been used extensively in the literature to describe any pathologic change that affects the renal allograft, the need to identify specific causes of CAN and understand the underlying mechanisms of allograft injury has been emphasized in recent years[17, 18]. The identification of such mechanisms is necessary to devise appropriate treatments and interventions for the long-term management of renal transplant patients.

Chronic damage and injury to renal allografts is mediated by alloimmune-dependent and alloimmune-independent mechanisms[19, 20]. One of the major alloimmune-dependent mechanisms of CAN is chronic rejection[17, 18]. Chronic rejection manifests as a slowly progressive cortical scarring of the allograft. This is mainly an antibody-mediated process as evidenced by the widespread C4d deposits (split product of the complement system classic pathway, marker of antibody-induced injury[21]) in the peritubular capillaries viewed by immunofluorescence microscopy[22, 23]. Human and animal studies have shown that donor-specific antibodies were associated with the development of CAN[24, 25]. Chronic, T-cell-mediated, allograft arteriopathy is also observed. This is suggested by the disruption of the lamina elastica interna, inflammatory cells in the fibrotic intima and proliferation of myofibroblasts in the intima[26, 27]. It is more likely, however, that chronic injury is mediated by an interplay of both the humoral and cellular arms of the immune system[28]. The role of calcineurin inhibitor (CNI) toxicity as a contributor to allograft damage has been recognized for several years[9, 29].

The exact mechanisms that cause allograft damage are not clearly understood but they all proceed through inflammatory pathways that involve the activation and upregulation

of different cytokines and adhesion molecules. For example, a recent animal study reported that an interleukin 6 (IL-6) -neutralizing antibody prevented occurrence of cyclosporine induced nephrotoxicity[30].

Other alloimmune-independent risk factors include older donor age[31, 32], younger recipient age[32], delayed graft function[33] and ischemia-reperfusion injury[34].

C. Periodontitis: association with systemic inflammation

Periodontitis is a chronic inflammation of the tooth-supporting tissues that is caused by an interplay between a sub-gingival, predominantly gram–negative, bacterial plaque/biofilm and an overt inflammatory response of a susceptible host [35, 36]. Periodontitis is characterized by progressive loss of the attachment and supporting bone of the tooth and, if left untreated, leads to eventual tooth loss.

The past decade has witnessed an explosion of information regarding a link between periodontitis and systemic diseases. Since several epidemiologic studies[37, 38] reported a significant association between periodontal disease and cardiovascular disease (CVD), the volume of research investigating a role for periodontitis in systemic inflammation and systemic disease, including stroke[39], diabetes mellitus[40], pre-term pregnancy[41] and end-stage renal disease[42] has been increasing exponentially.

In a meta-analysis of longitudinal and cross-sectional studies, Bahekar et al[43] reported that patients with periodontitis had a higher risk for developing CVD with a relative risk of 1.14 in prospective studies and odds ratio of 1.59 to 2.2 in case-control and cross-sectional studies. A randomized controlled interventional study[44] has shown that

intensive periodontal treatment improved endothelial function, a surrogate marker for future atherosclerosis[45], and reduced serum E-selectin levels at 2 and 6 months. This association, however, has not been confirmed in other studies[46, 47]. Other interventional studies are ongoing[48] and are expected to provide more information on the causality of the link and/or the benefit from periodontal treatment as measured by true endpoints of CVD.

A very interesting area of research in this field is to elucidate the mechanisms that explain the association between periodontitis and systemic inflammation and systemic diseases. One proposed mechanism is that many systemic diseases (e.g. atherosclerosis, diabetes mellitus) are in essence either induced or propagated by an inflammatory process[49, 50], and periodontal inflammation contributes incrementally to systemic inflammatory mediator levels. This would in turn contribute to the worsening or acceleration of the existing systemic disease. In support of this hypothesis, human studies reported higher serum C-reactive protein (CRP)[51, 52],(IL-6)[52], tissue plasminogen activator (TPA)[53], tumor necrosis factor alpha (TNF-α)[54, 55] in subjects with periodontal disease compared to periodontally-healthy subjects. While several studies reported a reduction of systemic cytokine levels after periodontal treatment[54, 56], other studies did not find significant reductions[57, 58]. Thus, further interventional studies with long-term follow-up are required to evaluate the benefit of periodontal treatment in modifying systemic inflammation and influence on systemic disease experience.

II. Periodontitis and Inflammatory Markers in Transplant Recipients

A. Objectives*:*

1. General objective:

To investigate whether inflammatory periodontal disease in solid-organ transplant recipients influences/contributes to systemic inflammatory cytokine levels, which might in turn affect the clinical course of the allograft.

2. Specific objectives:

Using a population of stable, solid-organ, transplant patients, and an age-matched systemically-healthy control population, we aimed to:

a) Investigate the prevalence of periodontal disease in a solid-organ transplant population and compare it to the prevalence of periodontal disease in a systemically-healthy control population.

b) Investigate if periodontal disease and different periodontal parameters are correlated with systemic interleukin 6 (IL-6) and C-reactive protein (CRP), after adjusting for known predictors/determinants of their systemic levels.

c) Investigate if IL-6 mRNA levels in gingival tissue from diseased sites are correlated with systemic levels of IL-6 in solid organ recipients.

B. **Introduction*:***

The link between periodontal inflammation and systemic health has been the focus of much scientific research in the past few years. This was stimulated by a better understanding of the role of inflammation in the pathogenesis of periodontitis and certain systemic diseases[49, 59, 60]. One proposed pathogenetic mechanism is that excessive release of inflammatory cytokines in diseased periodontal tissues contributes to the serum cytokine pool, which promotes systemic disease. Evidence supporting this hypothesis comes from *ex-vivo* and clinical studies. Cells within the diseased periodontium produce significantly higher levels of IL-6 compared to healthy gingival tissues, and periodontal disease has been linked to higher serum IL-6 in several studies[61, 62]. Similarly, serum CRP has been reported to be elevated in patients with periodontitis[63]. Perhaps, the most convincing evidence for a causal association between systemic and periodontal inflammation comes from studies showing that treatment of periodontitis reduced serum glycated hemoglobin and inflammatory marker levels in diabetic[64] or healthy subjects[44].

Solid organ transplantation is the definitive therapeutic approach in patients with end-stage organ disease. Kidney and heart are the most commonly transplanted organs, with more than 17,000 kidney and 2,000 heart transplants performed in the United States in 2008 (http://optn.transplant.hrsa.gov). Despite the high graft survival rates after the first year post-transplantation (more than 85%), the 10-year graft survival rates decrease dramatically (less than 55%)[65]. The main effector mechanism for chronic transplant deterioration is inflammation which is modulated by alloimmune-dependent and alloimmune-independent factors. Examples of alloimmune-dependent factors are human

leukocyte antigen (HLA) mismatches, panel reactive antibody (PRA) scores and acute rejection. Alloimmune-independent factors include cold ischemic time, ischemia/reperfusion injury, smoking, diabetes, and living versus cadaveric donor[20, 66]. Clinically, serum and urine IL-6 levels are indicators of acute rejection episodes[67, 68] and successful anti-rejection therapy reduces serum IL-6[69]. Similarly, CRP was shown to be a predictor of renal allograft survival[70].

Given the importance of IL-6 and CRP in transplant deterioration and the link between periodontitis and these systemic inflammatory markers, we explored whether periodontitis is a modifier of systemic IL-6 and CRP in solid organ transplant recipients. Thus, the goals of this study were: a) to assess the periodontal status of organ transplant recipients and compare it to an age-matched healthy group; b) to assess serum IL-6 and CRP levels and test a possible association between serum levels and gingival IL-6 mRNA expression.

C. Materials & Methods:

1. Subject recruitment

One hundred and forty-four renal and cardiac transplant recipients were screened during routine outpatient visits at Hartford Hospital Transplant Center. Inclusion criteria were: 1) clinically stable (measured by serum creatinine levels and cardiac ejection fraction in renal and cardiac transplant patients, respectively); 2) at least 1 year post-transplant; 3) absence of other systemic conditions that might directly affect systemic inflammatory status *e.g.* rheumatoid or autoimmune diseases; 3) negative history of antibiotic use

during the preceding 4 months; and 4) no periodontal treatment within the last year. Ninety renal and cardiac transplant patients met these criteria.

Recruitment strategy of controls included: 1) broadcast email to University of Connecticut Health Center (UCHC) employees; 2) Invitation letters mailed to General Clinical Research Center and Women's Health Center patients at UCHC; 3) Advertisements in local newspapers. Seventy-two systemically healthy subjects with no periodontal treatment within the last year and no history of antibiotic use during the preceding 4 months were recruited (36 UCHC employees, 16 invitation letter respondents and 20 newspaper advertisement respondents). The study was approved by the Institutional Review Boards of UCHC and Hartford Hospital.

2. Data Collection

Medical information of transplant subjects was extracted from hospital records and included age, gender, ethnicity, weight, height, diabetic status, smoking history, number of years since transplant, history of rejection episodes, medication regimen/dosages, HLA mismatching, PRA score, cadaveric or living donor, related or unrelated donor. A detailed health review questionnaire was used in the control group which included questions that might identify an undiagnosed systemic condition (e.g. frequency of urination, dermatologic lesions).

All subjects received a comprehensive oral examination including opportunistic infections, caries, missing teeth, plaque index (PI), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL) and gingival overgrowth (GO) according to Pernu et al[71]. Advanced periodontitis was defined as at least 2 interproximal sites with CAL≥6mm (not on the same tooth) plus 1 interproximal site with PD≥5mm[72].

3. Blood and Tissue Sampling:

Fifteen ml of venous blood were drawn from all subjects prior to oral examination. Gingival granulation tissue was collected from transplant patients with advanced periodontitis (n=19) who received non-surgical periodontal therapy, using a sharp curette in the deepest pocket. Gingival tissue samples were snap frozen in liquid nitrogen and stored at -80º C.

4. Sample analyses:

Within 2 hours of blood collection, sera were separated after clotting for 30 minutes at 4ºC, followed by centrifugation at 3,000 x g for 15 minutes. Aliquots were stored at -80ºC until testing. Sera were coded and analyzed in duplicate by *ELISA*[1]. The IL-6 and CRP assay analytical sensitivities were 2.0 pg/ml and <0.3 mg/L, respectively, and the variation in protein values within runs was <1% for both assays.

Total RNA was extracted from gingival samples[2] and quantitative real-time *PCR*[3] was performed using commercially available primers and probes[4] for IL-6. The *PCR* product

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[1] Diagnostic Products, Los Angeles, CA.

[2] TriZOL, Invitrogen, Carlsbad, CA.

[3] TaqMan Universal PCR Master Mix, Applied Biosystems.

[4] Applied Biosystems, Bedford, MA.

was measured in 40 consecutive cycles during each reaction using appropriate software[5]. IL-6 *cDNA* was normalized using 3 housekeeping genes (*GAPDH*, actin and $\beta_2 M$) in each sample. Nuclease-free water was used as a negative control in each reaction.

5. Statistical Analyses

Means and medians of continuous periodontal variables and percentage of sites with PD≥5mm or CAL≥4mm were calculated for each subject and group. Considering the frequency distribution of the values above the threshold of detection, undetectable serum IL-6 values were recorded as 0.5 pg/ml. Natural logarithm transformation was applied to non-normally distributed variables. Continuous and dichotomous variables were compared among groups using a two-sample student t-test or χ^2 test, respectively. Multivariate linear regression analysis determined predictors of serum IL-6 and CRP. Our transplant group sample size permits detection of an effect size of 0.18 at a p=0.05, in a multivariate analysis with 6 predictors and $\beta \leq 0.2$. Pearson correlation tested associations between serum IL-6, CRP and continuous periodontal variables. Partial correlations test was used to test the association between serum IL-6 and periodontal tissue IL-6 mRNA levels while controlling for diabetes. $p \leq 0.05$ was considered statistically significant.

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[5] iCycler iQ PCR detection system software, Bio-Rad.

D. Results

1. Population characteristics and periodontal findings

Population demographics, periodontal and serum findings in transplant and control groups appear in Table 1 (page 40) and Table 2 (page 42). Controls had slightly more females, lower body mass index (BMI) and lower percentage of diabetics compared to the transplant group. Mean PD, percentage of sites with PD≥5mm, percentage of sites with CAL≥4mm and number of missing teeth were higher in the transplant group compared to control. Twenty-one percent of the transplant subjects had advanced chronic periodontitis compared to 15% of the controls, but the difference was not statistically significant (p=0.34). Six and a half percent of the transplant group had mild localized gingival overgrowth (score 1), compared to 0% in the control group.

2. IL-6, CRP and periodontal status

The transplant group had higher mean serum IL-6 and CRP levels compared to the control group (Table 1, page 40). Serum IL-6 and CRP levels were positively correlated with each other, in transplant ($r=0.46$, $p<0.001$) and control groups ($r=0.35$, $p=0.003$). In the transplant group, there was no significant correlation between serum IL-6 or CRP levels and any of the periodontal indices, but the number of missing teeth showed a positive correlation with serum IL-6 ($r=0.29$, $p=0.006$).

Despite the lack of correlation between serum IL-6 and periodontal parameters, transplant patients with advanced periodontitis had a significantly higher serum IL-6 compared to non-periodontitis transplant subjects (6.0±4.2 pg/ml vs 4.7±4.4 pg/ml, p=0.04) and there was a trend towards higher serum CRP in this group as well (1.36

±1.89 mg/L vs 0.77±1.54 mg/L, p=0.12). Serum protein and gingival mRNA IL-6 levels were positively correlated in transplant subjects with advanced periodontitis, after adjusting for diabetes, but correlations were not statistically significant for any of the housekeeping genes (GAPDH: r=0.32, p=0.17; β_2M: r=0.28, p=0.23; actin: r=0.27, p=0.26).

In the control group, serum IL-6 levels were positively correlated with mean PD (r=0.37, p=0.002), percentage of sites with PD≥5mm (r=0.33, p=0.006) and mean BOP (r=0.38, p=0.001). Similarly in this group, serum CRP levels were positively correlated with mean PD (r=0.30, p=0.01) and CAL (r=0.29, p=0.01), whereas the correlation with the percentage of sites with PD≥5mm approached statistical significance (r=0.23, p=0.06). Differences in serum markers between advanced periodontitis and non-periodontitis control subjects did not reach statistical significance (3.7±1.3 pg/ml vs 2.9±1.6 pg/ml, p=0.06, and 0.38±0.42 vs 0.27±0.38, p=0.38, for IL-6 and CRP, respectively).

3. Predictors of serum inflammatory markers

In the transplant group, independent predictors of serum markers that were tested in the multivariate model were age, gender, diabetes (yes/no), smoking status (current/former vs. never smoker), BMI, years since transplant, immunosuppressant dose (prednisone, dichotomized at the 75th percentile), cyclosporine (yes/no), tacrolimus (yes/no), history of rejection (yes/no), living donor (yes/no), HLA mismatches, PRA score, advanced periodontitis, as well as all continuous periodontal variables. Variables with p value >0.2 were excluded from the model. The final model is shown in Table 3 (page 43) and Table 4 (page 44). Significant predictors of higher serum IL-6 in this group were older age, diabetes, higher BMI, and cadaveric donor, whereas the number of years post transplant

approached statistical significance. In the control group advanced periodontitis, higher BOP and greater percentage of sites with PD≥5mm were significant predictors of higher serum IL-6, whereas BMI approached statistical significance (Table 3, page 43). With respect to serum CRP levels, diabetes and higher BMI were significant predictors of higher CRP in the transplant whereas in the control group, only higher BMI was a significant predictor (Table 4, page 44).

E. Discussion

The prevalence of advanced chronic periodontitis in our transplant population was higher than the control group, but the difference was not statistically significant. The statistically significant differences we observed in certain continuous periodontal variables are clinically small and not unexpected considering that the transplant group had greater numbers of diabetics and former or current smokers, both of which are risk factors for periodontitis [73, 74]. Other studies, reported higher [75, 76], similar [77] or lower [78] prevalence of periodontitis in transplant subjects compared to controls. However, differences in periodontitis case definitions prohibit a direct comparison between studies.

In this study, periodontitis was defined using the AAP/CDC criteria for severe periodontitis [72]. This definition was chosen for several reasons: First, the definition is very stringent in its severe disease category, so that true disease can be detected outside the margin of measurement error. Second, this classification enables the identification of a population subset with a more pronounced and more readily identifiable systemic impact of periodontal inflammation [51, 56]. Third, the adoption of this definition by our study and, hopefully, future population-based studies will make the interpretation and comparison of data more feasible.

The transplanted organ constitutes a constant challenge to the host immune system. Chronic low-grade activation of inflammatory cells results in a cascade of events leading to elevated systemic inflammatory mediator levels [79, 80]. This explains our finding of elevated levels in circulating IL-6 and CRP in transplant subjects compared to healthy controls, as shown in earlier reports by our group and others [81, 82]

Interestingly, studies have reported that the exact sources of serum IL-6 are unclear and may originate in tissues other than the transplanted organ [83, 84]. While advanced periodontitis was a significant predictor of serum IL-6 in the control group, it was not a significant predictor in the transplant group ($F=0.06$, $p=0.8$). Significant predictors of higher IL-6 were older age, diabetes, higher BMI and a cadaveric donor, consistent with other reports in the literature [85-88].

The lack of significance in the multivariate analysis between periodontal parameters and inflammatory markers in transplant subjects, which contradicts some of our preliminary findings [82], could be due to several reasons. A possible explanation is a true lack of association, given the strong “inflammatory” confounding by diabetes and the constant allograft-mediated antigenic stimulation in transplant subjects. Another reason could be that periodontally-driven inflammation is dampened by immunosuppressive therapy, which is supported by the finding that transplant patients experience limited progression of periodontitis after initiation of immunosuppression [77].

The positive correlation between serum and gingival IL-6 in transplant patients, consistent with our preliminary findings [82], is noteworthy. Although only having a trend

for statistical significance, this moderate size correlation, considering the small sample size and possible effects of immunosuppressive treatment on the periodontium, suggests a possible contribution of periodontal tissue IL-6 to circulating IL-6. Nevertheless, clinical periodontal indices were not predictive of serum IL-6 in the transplant population. These seemingly conflicting findings could be reconciled by acknowledging that periodontitis levels were relatively low in our transplant population, which might have prohibited finding a significant association with serum IL-6 levels. Additionally, gingival mRNA levels were only quantified in the deepest pockets of a transplant subset with advanced periodontitis whereas clinical periodontal indices were assessed in all patients.

The cross sectional nature of this study prevents the assessment of a cause and effect relationship between periodontitis and systemic inflammation. Future research should aim at longitudinal evaluation of periodontal indices and their correlation with systemic markers of inflammation, as well as the influence of periodontal treatment on local and systemic markers of inflammation in this population.

III. Periodontal Disease, Renal Function and Chronic Allograft Nephropathy

A. Objectives*:*

1. General objectives:

To investigate if clinical and/or histological renal allograft deterioration is associated with periodontal disease severity, and to determine whether the choice of clinical determinants of periodontal disease can affect statistical outcomes.

2. Specific objectives:

Using a population of stable renal transplant patients:

a) To investigate if deterioration in glomerular filtration rates (GFR) is associated with periodontal disease severity or various periodontal parameters.

b) To investigate if biopsy-proven Chronic Allograft Nephropathy (CAN) is associated with prevalence of periodontitis, or clinical indices of severity of periodontitis.

c) To investigate whether different periodontitis case definitions modify the resulting associations with GFR changes or biopsy-proven CAN.

B. Introduction:

More than 16,500 kidney transplants were performed during 2008 across the US (http://optn.transplant.hrsa.gov). Despite greatly improved 1-year survival rates of renal transplants from living and deceased donors (90 and 95%, respectively), the 10-year graft survival rates fall dramatically to 46 and 58%, respectively[89]. In renal transplants, 50–80% of these late failures are attributable to chronic allograft nephropathy (CAN) [89, 90] which is considered the most important cause of renal graft failure after the first year of transplantation[90, 91]. CAN is a descriptive term for a number of histologic lesions in renal transplants characterized by progressive interstitial fibrosis, glomerulopathy, mesangial matrix increase, vascular fibrous intimal thickening and arteriolar hyaline thickening[20]. Clinically, CAN presents as progressive deterioration in renal function, proteinuria, and occasionally, de novo or secondary hypertension.

Several alloimmune-dependent and alloimmune-independent factors are believed to influence the development of these histologic changes. Examples of alloimmune factors include acute rejection episodes, subclinical rejection, HLA mismatching and allosensitization. Alloimmune-independent factors include graft ischemia, donor age, cytomegalovirus infection, brain death, calcineurin inhibitors, recipient morbidity, smoking, diabetes mellitus, age, obesity and hyperlipidemia[20]. These different contributing factors are thought to result in transplant endothelial cell injury and activation, which is considered to be a key event in the pathogenesis of CAN. Endothelial cell activation triggers a cascade of complex and interdependent events. The activated endothelium secretes an array of inflammatory cytokines (interleukin 6 (IL-6),

interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-α)) leading to progressive stimulation of other inflammatory cells (e.g. macrophages, T-lymphocytes). This in turn triggers upregulation of adhesion molecules such as ICAM's, selectins and MHC molecules. Thus, leukocyte infiltration is promoted and further cytokine release takes place from activated inflammatory cells leading to phenotypic switching of key effector cells (glomerular mesangial cells, fibroblasts, smooth muscle cells). These series of processes culminate in the accumulation of extracellular matrix proteins in the interstitium and intima of the vascular endothelium. A vicious circle of sustained inflammatory reaction from the host's immune system to the allograft is established, and leads to progressive graft destruction and loss of function. Interestingly, a heightened pre-transplant or post-transplant systemic inflammation, as measured by a multitude of serum cytokines (e.g. C-reactive protein (CRP), vascular adhesion molecule-1, interleukin 12) has been associated with worse renal allograft outcomes including acute and chronic rejection[70, 92-95].

An oral inflammatory disease that received much attention recently due to a possible link with systemic inflammation is chronic periodontitis. Chronic periodontitis and consequent destruction of periodontal tissues is believed to be caused by an interplay between a sub-gingival, predominantly Gram–negative, bacterial plaque/biofilm and an overt inflammatory response of a susceptible host[35, 36]. Many studies have attempted to dissect the determinants of host susceptibility to periodontal disease[96-99]. For example, epidemiological studies showed that the variability in the level of plaque control within a population is not always commensurate with disease prevalence[100, 101]. Moreover, molecular analysis of microbial flora revealed a weak positive predictive value for the presence or absence of specific periodontal pathogens and disease activity[100-102].

Several studies have also shown a hyper-active phagocytic cell phenotype in chronic periodontitis patients[103, 104], in which excessive production of inflammatory cytokines, proteases and generation of higher levels of reactive oxygen radicals result in destruction, as opposed to a protection[103, 104]. Cumulatively, this evidence indicates that host susceptibility to periodontitis is related to a hyper-inflammatory phenotype leading to an exaggerated inflammatory response to microbial challenge and subsequent untoward destruction of the attachment apparatus.

There is accumulating evidence that links periodontal disease to various systemic diseases and conditions, including diabetes mellitus[40, 105], cardiovascular disease[43] and poor pregnancy outomes[106-108]. Moreover, higher systemic inflammatory cytokine levels, such as CRP, IL-6 and E-selectin, have been associated with periodontal disease[44, 51, 57, 62, 63] and significant reductions were seen after periodontal treatment[44, 54, 109]. These findings, however, were not confirmed in other studies[57, 110-112]. Interestingly, a recent study[113] reported that the criteria used to diagnose/define a case of periodontitis has a significant impact on the resulting associations between periodontitis and pre-term, low-birth weight pregnancy. The significance of the definition applied to diagnose a case of periodontitis is also apparent when large epidemiologic studies are compared[72], since some of the observed trends in disease prevalence and related associations are merely due to different disease thresholds being applied.

Given the evidence that links periodontitis to a hyper-reactive inflammatory phenotype[103, 104] and higher systemic inflammatory cytokine levels[9, 51, 63], we hypothesized that periodontal disease could serve as a marker to identify subjects with a systemic hyper-inflammatory phenotype that may consequently be at greater risk for long-term renal

allograft deterioration. This would have several implications. For example, a history of periodontitis may identify a population in need of more strict criteria in HLA-matching before transplantation. This could also necessitate closer monitoring of periodontitis-susceptible individuals after renal transplantation for signs of CAN and additional graft biopsies might be indicated. Finally, in as much as chronic periodontitis can affect systemic inflammatory cytokine levels, this oral infection could also be causally linked to CAN, although the cross-sectional nature of our study cannot test this hypothesis. The aim of this analysis is to investigate the association between the periodontal status of a cohort of renal transplant patients and renal allograft function and biopsy-proven CAN. Additionally, considering the reported impact of periodontal disease definition on the observed associations with systemic disease, we investigated the impact of applying three different periodontitis case definitions on the observed associations.

C. Materials and methods:

1. Subject recruitment

For the purposes of this analysis, a subset of the population described in chapter 2 was included. This subset was defined by the following criteria: 1) clinically-stable renal transplant subjects; 2) at least 1 year post-transplant; 3) absence of other systemic conditions that might directly impact the systemic inflammatory status (e.g., rheumatoid or autoimmune diseases); 3) negative history of antibiotic use during the preceding 4 months; and 4) no periodontal treatment within the last year; 5) availability of data on the highest and lowest serum creatinine tests on record, with these tests being at least 6 months apart and at least 6 months after the transplant. Fifty-eight renal transplant patients who met these criteria were included.

2. Data Collection

Medical information of the subjects was extracted from medical records. A standardized extraction form was used. The extracted data included: patient demographics (age, gender, ethnicity, weight, and height), co-existing systemic conditions, e.g. diabetes (yes/no), hypertension (yes/no), smoking status (current, former and never smoker), number of years since transplant, history of rejection episodes, medication regimen and dosages, graft survival risk factors (e.g. human leukocyte antigen (HLA) mismatching, panel reactive antibody (PRA) score, cadaveric or living donor (related or unrelated), delayed graft function, cold ischemic time, dialysis before transplant, cytomegalovirus infection), serum creatinine lab results and allograft biopsy reports if available.

Using the highest and lowest serum creatinine values for each subject, GFR values were estimated using the 4-variable simplified MDRD formula [114] (estimated GFR=186 x Serum Creatinine $^{-1.154}$ x Age $^{-0.203}$ x [1.210 *if Black*] x [0.742 *if Female*]). Subjects were divided into two groups based on whether a deterioration or improvement of GFR occurred over time (subjects with no change were included in the latter group). In addition, using the Banff 1997 classification[27], the subjects were classified according to the degree of chronic allograft nephropathy as determined in available biopsy reports. The criteria for the different categories of this classification are shown in Table 5 (page 45).

Subjects received a comprehensive oral examination. The following parameters were evaluated and recorded: soft tissue lesions, opportunistic infections commonly associated with immunosuppression, caries, missing teeth (excluding third molars),

plaque score (PS), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL) and gingival overgrowth (GO) based on the gingival overgrowth index by Pernu et al[71]. Chronic periodontitis was defined using 3 different definitions: the first definition (DEF1) was 2 or more inter-proximal sites with PD≥5mm or CAL≥4mm (not on the same tooth). The second definition of chronic periodontitis (DEF2) was 6 or more inter-proximal sites with PD≥5 or CAL ≥ 4. The third definition of chronic periodontitis (DEF3) was 2 or more inter-proximal sites in each quadrant with a PD≥5 or CAL ≥ 4. These definitions were chosen because DEF1 comprises of the moderate and severe periodontitis categories of the AAP/CDC periodontitis case definition[72], and DEF2 and DEF3 are arbitrary definitions that were devised to represent an escalating extent of periodontal destruction compared to DEF1.

3. Blood sampling and analyses:

Fifteen ml of venous blood were withdrawn from all subjects prior to oral examinations. Serum IL-6 and CRP were measured using enzyme-linked immunosorbent assay. Techniques were described in detail in chapter I (page 12).

4. Statistical analyses:

A natural log transformation was applied to non-normally distributed variables. Means and medians were calculated for continuous variables. Student t-test was used to test for differences between the groups (GFR deterioration vs. improvement). A multivariate, repeated measures, linear regression analysis was applied to determine significant predictors of the within-subject change in GFR values over time. Student t-test was used

to test for differences of the periodontal variables between the different Banff CAN score groups. A p value ≤0.05 was considered statistically significant.

D. **Results*:***

The population demographic and medical data are reported in Table 6 (page 46). Diabetic patients comprised 52% of the subjects and 53% were former or current smokers. The median number of years post-transplant was more than 7 years. The mean time between the two serum creatinine tests was 44.7±43 months with a range of (6-200) months. 60% of the subjects had a deterioration of estimated GFR levels with a median value of GFR deterioration of 50 ml/min/1.73 m^2 (Table 7, page 47). Serum IL-6 and CRP were not statistically significantly different among the GFR deterioration vs. improvement/stable groups but there was a trend for higher serum IL-6 and CRP in the GFR improvement (Table 8, page 48).

Periodontal parameters in the GFR improvement vs. deterioration groups are reported in Table 9 (page 49). There were no statistically significant differences in mean PD, mean CAL, percentage of sites with BOP, plaque score, percentage of sites with PD≥5mm, percentage of sites with CAL≥4mm or the number of missing teeth among the two groups. The prevalence rates of chronic periodontitis in the GFR improvement vs. deterioration groups according to the 3 different definitions used are described in Table 10 (page 50). There were no significant differences in the prevalence of periodontitis between the two groups when DEF 1 and DEF3 were used. However, using DEF2, chronic periodontitis was more prevalent in the GFR deterioration group and the difference was statistically significant (Fisher's exact test, p=0.02) (Table 10, page 50).

The serum IL-6 and CRP levels in subjects with and without periodontitis according to the 3 different definitions are shown in Table 11 (page 51). No statistically significant differences were observed.

A multivariate, repeated measures, linear regression analysis was conducted to determine significant predictors of the GFR change over time. The variables included were body-mass index (BMI), diabetes status (yes/no), smoking history (yes/no), living or cadaveric donor, hypertension, dialysis before transplantation, cold ischemic time, delayed graft function, acute rejection episodes (yes/no), panel reactive antibody score, HLA mismatch (less than 3 vs. 3 mismatches or more), cytomegalovirus infection (yes/no), prednisone use (yes/no), cyclosporine use (yes/no), serum IL-6 and serum CRP. Continuous periodontal parameters such as mean PD, mean CAL, percentage of sites with BOP, percentage of sites with PD≥5mm, percentage of sites with CAL≥4mm and the number of missing teeth were included individually in the model (i.e. they were not tested simultaneously). Chronic periodontitis, as defined by each definition was included in the model individually. Variables that returned a p value >0.2 were excluded from the final model. Accordingly, the following variables were excluded from the final analysis: BMI, diabetes status (yes/no), smoking history (yes/no), dialysis before transplantation, cold ischemic time, delayed graft function, panel reactive antibody score, HLA mismatch (less than 3 vs. 3 mismatches or more, cytomegalovirus infection, prednisone use (yes/no), cyclosporine use (yes/no), serum IL-6 and serum CRP. The model which includes the significant medical predictors is shown in Table 12 (page 52). The direction of change predicted by each of these variables can be deduced by examining the estimated means of GFR within the categories of each variable, shown in Table 13 (page 53). GFR change over time (the dependent variable) was statistically

significant (F=6.12, p=0.02; this represents the impact of time between the two serum creatinine measurements on GFR levels within the same subject). History of acute rejection, having a living donor, and being hypertensive statistically significantly predicted deterioration of renal allograft function. When the continuous periodontal variables were introduced individually into the model, the percentage of sites with BOP, mean PD and the percentage of sites with PD≥5mm showed a trend towards statistical significance in predicting GFR change (Table 14, page 54). To determine the direction of change predicted by each of these variables (percentage of sites with BOP, mean PD and the percentage of sites with PD≥5mm), the variables were re-coded into a trichotomy with cutoff points at the 25th and 75th percentiles. The estimated means of GFR within the categories of each of these variables are shown in Table 15 (page 55). Subjects with higher percentage of sites with BOP or PD≥5mm, or a higher mean PD, had a trend for greater GFR deterioration. Other continuous periodontal variables were not statistically significant in the model (mean CAL: F=0.06, p=0.81; percentage of sites with CAL≥4mm: F=0.74, p=0.39; number of missing teeth: F=0.15, p=0.70).

The 3 different periodontitis definitions as were included individually included in the model. Chronic periodontitis as defined by DEF2 emerged as a statistically significant predictor of GFR deterioration, while DEF1 and DEF3 were not statistically significant (F=0.05, p=0.83; F=0.89, p=0.35, respectively). The direction of change predicted by chronic periodontitis DEF2 can be deduced by examining the estimated means of GFR in subjects with and without chronic periodontitis, shown in Table 17 (page 58).

Twenty-two subjects had a needle-core biopsy report available. Information regarding the different time spans between the date of the transplant, date of biopsy, date of the

GFR measurements and the date of oral exam are shown in Table 18 (page 59). Most of the subjects had the biopsy taken within one year of the most recent GFR available. One subject, however, had the biopsy done more than 5 years before the date of the most recent GFR, which explains the skewed distribution and the large standard deviation observed. Based on the biopsy report, The BANFF CAN score was deduced for each subject. Percentage distribution of each score among the 22 subjects is shown in Figure 1 (page 63). Due to the small sample size, the CAN score variable was dichotomized into a CAN group (Banff CAN scores 1, 2 and 3) vs. no CAN group (Banff CAN score 0).

All 22 subjects that had a biopsy were from the GFR deterioration group. A student t-test was applied to examine if there were differences in the continuous periodontal variables (mean PD, mean CAL, percentage of sites with PD≥5mm, percentage of sites with CAL≥4mm, percentage sites BOP, number of missing teeth) among the two CAN score groups. There were no statistically significant differences among the two groups for any of the periodontal variables. A chi square test was used to examine any significant association between chronic periodontitis history (using the three different definitions) and the severity of CAN. No significant associations were found for all three definitions (according to DEF2, all but one subject had chronic periodontitis, which curtails statistical analysis).

E. Discussion

This secondary analysis of data was performed on a subset of the renal allograft recipient population reported in chapter (2). The subjects were included in the analysis if the available creatinine lab results were taken at least 6 months after the transplantation

and the two measurements were 6 months apart. Those cutoff points were chosen since one large study showed that allograft function at 6 months post-transplant, as measureby creatinine values, and the changes that occurred 6 months later were related to the 5-year survival of the graft.[115] In our study, the GFR values were estimated using the simplified MDRD formula. The simplified MDRD formula showed comparable accuracy and correlation to other MDRD formulas in estimating GFR in renal transplant recipients, and better prediction of true GFR compared to the Cockcrof & Gault formula.[116, 117]

Conventionally, graft failure is suspected only when a continued and irreversible fall in renal function has become clinically apparent, generally, as a combination of hypertension and proteinuria. Subsequently, renal allograft biopsies are performed to identify acute graft dysfunction[118]. Recent studies have indicated that acute rejection episodes and CAN are often subclinical without any significant impairment of renal function[119-121]. These observations have sparked the debate regarding the validity and benefit of protocol/surveillence biopsies for stable renal allograft patients with the objective of early intervention in case of graft deterioration[118].

The success that has been achieved in improving early survival rates of renal transplants is still over-shadowed by a significant drop in long-term patient and graft survival after 10 years.[7] Several parameters have been shown to be correlated with long-term allograft outcomes such as acute rejection episodes,[122] the number of human leukocyte antigen (HLA) mismatches,[123] delayed graft function,[124] and having a living vs. a cadaveric donor.[125] Nevertheless, these parameters can't explain the variation in the clinical course and outcome of organ transplantation.

A considerable body of research has focused on the role of genetic polymorphism in organ transplantation[126-128]. The primary hypothesis of such research is that genotypic differences lead to different phenotypes of immune responsiveness which is related to variable inflammatory cytokine, and other inflammatory molecule synthesis. Those differences are in turn determined by gene polymorphisms. Several cytokine gene polymorphisms have been examined, including INF-γ[129-131], TNF-α[131, 132], IL-10[130] and IL-6[133], but associations with graft survival were not conclusive[132] and often appeared contradictory[130, 131, 133]. Other mediator molecules investigated, with similar inconsistent findings, include growth factors, e.g. TGF-β[130, 132], adhesion molecules (e.g. ICAM)[134], and co-stimulatory molecules (e.g. CCR5)[130, 135]. Similarly, susceptibility to periodontitis has been hypothesized to be related to various gene polymorphisms. Researchers have investigated polymorphisms involving IL-1[96, 136], IL-6[98], TNF-α[98, 137], vitamin D receptor[138], TLR4[98, 139] and others[140], but the associations with chronic or aggressive periodontitis were not consistent[141, 142]. The inconsistent findings are possibly due to the complex inflammatory-mediator interactions, whereby a single nucleotide polymorphism is unlikely to explain the spectrum of variation observed in either or both CAN or periodontitis. Thus, our hypothesis is based on a broad assumption whereby a subjects' "pro-inflammatory trait/phenotype" is inferred from periodontal disease experience, which in turn might predict worse long-term allograft outcomes.

In this analysis, we proposed that history of periodontitis indicates a hyper-inflammatory phenotype, which could in turn cause greater susceptibility to renal allograft deterioration. This scheme of interaction does not necessarily assume causality, and could be extended to other areas where a link between periodontitis and various systemic

diseases is being investigated. In this analysis, periodontal destruction was measured using a series of continuous variables and 3 different “periodontitis case” definitions. The decision to include these 3 case definitions was triggered by a recent study[113] that showed that when different disease definitions were applied in the same patient population, considerably different associations were found when periodontitis and systemic diseases are investigated.

Sixty percent of our patient cohort experienced a deterioration of GFR function by a median change of 48 mL/min/1.73m^2, while 40% experienced a median improvement of 44 mL/min/1.73m^2. These numbers are generally in agreement with observations in other studies[143, 144], with similar populations that compared renal function measures over time. At least 50% of the patient cohort had the two serum creatinine tests within 2.3 years. The trend toward higher cytokine levels in the GFR improvement group might be considered counter-intuitive. Interestingly, Muller and colleagues found that a high-expression IL-6 genotype was associated with better graft survival at 3 years. [145]

Continuous periodontal variables were not statistically significantly different between the GFR groups. Clinically-small trends were, however, observed for greater periodontal destruction and inflammation in the GFR deterioration group as measured by the percentage of sites with BOP, mean PD and percentage of sites with PD≥5mm. When the 3 different definitions were used to classify the subjects into periodontally-healthy or -diseased subjects, varying prevalence rates were observed. While periodontitis was more prevalent in the GFR deterioration group compared to the GFR improvement group according to the 3 definitions, the difference was statistically significant only when using DEF2. These findings underscore the importance of the “case definition of periodontitis”

in any study, especially studies investigating a link between periodontitis and systemic diseases. This is further highlighted by the fact that even when the differences between the criteria to define disease were small by clinical standards (DEF2 requires 6 proximal sites with PD≥5 or CAL ≥ 4 while DEF3 requires 8 sites), this was enough to have a bearing on the statistically significant associations found.

In the multivariate analysis, the demographic and medical variables that were statistically significantly associated with GFR deterioration were history of acute rejection, having a living donor, and being hypertensive. Although history of acute rejection[146, 147] and hypertension[144] have been shown to be associated with allograft deterioration, renal allografts from living donors have higher survival rates compared to allografts from cadaveric donors (http://optn.transplant.hrsa.gov). Our finding of greater renal function deterioration in subjects with a renal transplant from a living donor could not be explained by examining the differences in the number of HLA mismatches, percentages of related and unrelated donors or number of episodes of acute rejection between transplant patients with a living vs. a cadaveric donor (Table 19, Table 20 and Table 21; pages 60-62). Chronic periodontitis as defined by DEF2 was statistically significant in predicting GFR deterioration. Additionally, the continuous periodontal variables were introduced individually into the model, higher percentage of sites with BOP, higher mean PD and higher percentage of sites with PD≥5mm showed a trend towards statistical significance in predicting deterioration of allograft function (percentage sites BOP: F=3.27, p=0.08; mean PD: F=3.02, p=0.09; percentage of sites with PD≥5mm: F=2.28, p=0.14). These findings suggest that periodontal destruction might be a risk indicator for renal allograft deterioration. Although the analysis of the biopsy report did not yield any

significant findings, this might be due to the variation in the time period between the biopsy exam and the oral exam in this study, as well as the small sample size.

Our analysis has several limitations. The GFR values are estimated from two creatinine measurements that were done at different time intervals for each patient. Also, the most recent serum creatinine measurement was not done at the same time of the oral examination. In addition, the single periodontal examination performed in this study does not permit accurate evaluation of the activity of the disease, although, our hypothesis addresses the existence of a susceptible phenotype as a risk indicator and not the causality of the link between active periodontitis and graft deterioration. Another limitation is that the timing of the biopsy was very variable in relation to the timing of the creatinine tests available (some biopsies were done before, between or after the creatinine lab tests). Finally, in the multivariate model, adjusting for medications introduced bias because patient records reveal that many subjects had frequent changes to their medication regimen.

In conclusion, although our findings are suggestive of the potential for “periodontitis susceptibility” to predict renal allograft deterioration, no definitive conclusions can be drawn. Nevertheless, considering that this was a secondary analysis of cross-sectional data, and given the small sample size, this study could serve as a pilot study for power calculations in a future study. A longitudinal study design is necessary that would include subjects that receive an oral examination pre-transplantation, and whereby serial creatinine measurements, protocol biopsies and prospective monitoring of allograft function and periodontal status are conducted. Also, an intervention could be included in this design in which periodontitis patients would receive intensive periodontal treatment

pre- or post-transplant. These types of studies could not only support biologically plausible associations but also can demonstrate causality.

IV. Concluding Remarks

1. Advanced periodontitis was found in 21% of the subjects in a population of stable renal and cardiac transplant patients. This was not statistically significantly different from prevalence in a control group (15%).
2. Advanced periodontitis did not emerge as a statistically significant predictor of serum IL-6 or CRP in renal and cardiac transplant patients after adjusting for known modifiers of these cytokines.
3. Serum IL-6 was moderately and positively correlated with gingival IL-6 mRNA expression, although this was a statistical trend only.
4. Periodontal disease and periodontal inflammation, as measured by percentage of sites with BOP, mean PD and percentage of sites with PD≥5mm, indicated risk for renal allograft deterioration as measured from estimated GFR values (β=0.25, 0.24, 0.22, respectively) .
5. Periodontal parameters did not correlate with the chronic histologic changes observed in renal allograft biopsies. This analysis, however, involved only a small subset of the population.
6. In studies evaluating the association between periodontitis and systemic diseases or conditions, the criteria used to define a periodontitis case have a significant influence on the resulting associations.

V. Index (tables and figures)

Table 1: Characteristics of transplant and control groups:

Variable	**Transplant group (n=90)**		**Control group (n=72)**		**P value***
	Mean± SD	Median (quartiles)	Mean± SD	Median (quartiles)	
Age (years)	53 ± 12	53.5 (44, 61)	51 ± 12	51 (43, 59)	0.33(NS)
Gender (female) (%)	42%		60%		0.027
Diabetics (%)	56%		3%		<0.001
Smokers (current and former) (%)	31%		0%		<0.001
BMI	30.2 ± 7.2	29.3 (24.5, 33.5)	27.3 ± 5.0	26.2 (24.0, 30.6)	0.007
PD (mm)	2.7 ± 0.5	2.6 (2.4, 3.0)	2.4 ± 0.4	2.4 (2.2, 2.7)	0.001
CAL (mm)	2.9 ± 0.7	2.8 (2.5, 3.2)	2.7 ± 0.6	2.6 (2.3, 3.0)	0.06 (NS)
BOP (%)	17.0 ± 16.7	11.9 (4.8, 22.5)	22.0 ± 16.7	17.0 (8.7, 34.0)	0.06 (NS)
PI (%)	46.6 ± 30.0	45.9 (19.1, 71.7)	49.0 ± 28.5	53.3 (22.9, 73.5)	0.60 (NS)
No. of missing teeth **	4.2 ± 4.9	3 (1, 6)	1.7 ± 2.2	1 (0, 3)	<0.001

% Sites with CAL≥ 4mm	14.4 ± 16.9	8.7 (2.8, 18.7)	9.6 ± 13.5	3.8 (0.0, 14.3)	0.046
% of sites with PD≥ 5mm	5.2 ± 7.9	1.3 (0.0, 8.3)	2.6 ± 5.2	0.0 (0.0, 2.6)	0.013
Advanced periodontitis (%)	21%		15%		0.34
IL-6 (pg/ml)	5.0 ± 4.3	3.3 (2.4, 5.1)	3.0 ± 1.6	2.5 (2.0, 3.7)	<0.000
CRP (mg/L)	0.9 ± 1.63	0.30 (0.10, 0.82)	0.28 ± 0.39	0.12 (0.05, 0.28)	<0.000

SD: standard deviation, NS: not significant

* t-test or chi square

** 3rd molars excluded

Table 2: Medical and demographic data of the transplant population

	Transplant subjects (n=90)*
Transplant type (kidney)	90%
Ethnicity (black)	32%
Post-transplant years	6.9 ± 4.8, 5.0(3.0, 10.0)
History of acute rejection	36%
Pretransplant dialysis	68%
Living donor (related or unrelated)	42%
Cyclosporin	24%
Gingival overgrowth	6.0%
Ca++ channel blockers	29%
Prednisone	87%
Mycophenolate	72%
Tacrolimus	54%
Azathioprine	10%
Sirolimus	7%

* Continuous variables shown as mean ± standard deviation, median (quartiles) and categorical variables reported as percentage frequency

Table 3: Multivariate linear regression analysis with serum IL-6 as the dependent variable

Transplant group*	Variable	F	p value	β
	Age	7.93	<0.01	0.30
	Diabetes history	6.30	<0.02	0.27
	BMI	7.10	<0.01	0.29
	Cadaveric donor	6.21	<0.02	0.27
	Number of years post-transplant	3.00	0.09	0.19
	Number of missing teeth	3.52	0.06	0.21
Control group**	Variable	F	p value	β
	Gender	0.002	0.96	<0.01
	Age	0.42	0.52	0.08
	Diabetes history	0.20	0.66	0.05
	BMI	3.56	0.06	0.24
	Advanced periodontitis	6.51	0.01	0.31
	percentage sites with BOP	10.40	<0.01	0.39

* $r^2 = 0.30$

** $r^2 = 0.26$

Table 4: Multivariate linear regression analysis in the transplant group with serum CRP as the dependent variable

Transplant group*	Variable	F	p value	β
	Age	2.31	0.13	0.16
	Diabetes_Hx	8.48	0.005	0.30
	BMI	11.66	0.001	0.35
	Advanced periodontitis	1.56	0.22	0.14
Control group*	Variable	F	p value	β
	Age	0.98	0.33	0.13
	Diabetes_Hx	0.81	0.37	0.11
	BMI	14.23	<0.001	0.43
	Advanced periodontitis	0.09	0.76	0.04

* $r^2 = 0.23$

** $r^2 = 0.21$

Table 5: Banff 97 Classification of Chronic Allograft Nephropathy in Biopsies

Chronic Allograft Nephropathy	
Grade	Histopathological findings
1 (mild)	Mild interstitial fibrosis and tubular atrophy without or with specific changes suggesting chronic rejection
2 (moderate)	Moderate interstitial fibrosis and tubular atrophy
3 (severe)	Severe interstitial fibrosis and tubular atrophy and tubular loss

Table 6: Demographic and Medical Characteristics

Variable	Mean ± sd (frequency)	Median
Age	52.8±12.0	53.3
Gender (female) (%)	41%	
Race (black)	24%	
Diabetics (%)	52%	
Smokers (current and former) (%)	31%	
Yrs after transplant	7.6±4.9 (n=58)	7.1 (3.8, 11.0)
History of acute rejection	33%	
Pre-transplant dialysis	78%	
Living donor (related or unrelated)	43%	
Ca++ channel blockers	28%	
Cyclosporin	38%	
Prednisone	91%	
Mycophenolate	72%	
Tacrolimus	57%	
Azathioprine	21%	
Sirolimus	14%	

Table 7: Estimated GFR Measurement Time Intervals and GFR Change:

Variable	Mean ± SD (frequency)	Median (quartiles)
Months between two GFR tests	44.7±43.1	Range (6-200) Median 28.5 (13.8, 64.7)
Months between oral exam and recent GFR	26.7±24.4	Range (2-123) median 16.7 (11.3, 34.2)
GFR deterioration (% subjects)	60%	
GFR Deterioration (mL/min/1.73 m^2)	56	48 (66, 36)
GFR improvement (% subjects)	40%	
GFR Improvement (mL/min/1.73 m^2)	44	44 (26, 56)

Table 8: Serum IL-6 and CRP cytokine levels in the GFR groups:

	GFR improvement group (mean ± SD)	GFR deterioration group (mean ± SD)	p-value *
IL-6 (pg/ml)	5.9 ± 2.0	4.1 ± 1.8	p=0.06
CRP (mg/L)	0.92 ± 2.10	0.44 ± 1.50	p=0.11

* Student t test

Table 9: Continuous Periodontal Variables in the GFR Groups:

Variable	GFR deterioration		GFR improvement		P value*
	Mean±SD	Median (25^{th}, 75^{th} quartiles)	Mean±SD	Median (25^{th}, 75^{th} quartiles)	
Mean PD (mm)	2.8 ± 0.4	2.7 (2.4, 3.2)	2.6 ± 0.5	2.6 (2.4, 3.0)	0.25 (NS)
Mean CAL (mm)	3.0 ± 0.8	2.8 (2.5, 3.3)	2.9 ± 0.7	2.7 (2.4, 3.4)	0.72 (NS)
BOP (% of sites)	18.8 ± 15.5	15.0 (6.5, 25.0)	14.7 ± 13.8	7.7 (4.6, 28.5)	0.14 (NS)
PI (% of sites)	50.7 ± 29.0	53.6 (28.3, 76.9)	43.0 ± 33.0	42.4 (10.7, 74.4)	0.35 (NS)
No. of missing teeth **	2.6 ± 2.6	2.0 (1.0, 4.0)	4.5 ± 4.5	4.0 (0.0, 8.0)	0.29 (NS)
Sites with CAL≥4mm (%)	16.6 ± 20.1	9.1 (3.1, 19.2)	14.9 ± 19.8	3.0 (1.8, 20.8)	0.30 (NS)
Sites with PD≥5mm (%)	7.0 ± 9.2	2.4 (0.0, 12.3)	5.6 ± 9.0	0.0 (0.0, 11.4)	0.22 (NS)

** excluding 3^{rd} molars

Table 10: Prevalence of Chronic Periodontitis in the GFR Groups:

Chronic periodontitis (prevalence in each group)	GFR deterioration	GFR improvement	P value
DEF 1	94%	87%	p=0.44 * (NS)
DEF 2	91%	70%	p= 0.04 *
DEF 3	57%	39%	p=0.18 ** (NS)

* Fisher's exact test

** Chi square test

Table 11: Serum IL-6 and CRP in periodontitis vs. non-periodontitis subjects (mean ± standard deviation)

	DEF1		
	CP*	No CP	p value
Serum IL-6	5.1 ± 1.9	4.6 ± 1.9	0.76
Serum CRP	0.60 ± 1.8	0.45 ± 1.5	0.66
	DEF2		
	CP	No CP	p value
Serum IL-6	4.7 ± 1.9	4.3 ± 1.7	0.70
Serum CRP	0.68 ± 1.85	0.36 ± 1.34	0.11
	DEF3		
	CP	No CP	p value
Serum IL-6	4.6 ± 1.8	4.7 ± 2.0	0.90
Serum CRP	0.60 ± 1.7	0.60 ± 1.8	0.75

* CP: chronic periodontitis

Table 12: Multivariate, repeated measures, linear regression* with GFR change over time as the dependent variable (statistically significant medical variables):

	F	p value
GFR change over time (dependent variable)	6.23	.016

Independent variables	F	p value	β	Partial eta squared	Observed Power
History of acute rejection	4.80	.033	0.30	0.09	0.58
Living vs cadaveric donor	6.53	.014	0.34	0.12	0.71
Hypertension	4.01	.051	0.28	0.08	0.50

* n=53

Table 13: Estimated means of first (older) and second (recent) GFR within the categories of the significant variables:

Variable	GFR (Mean ± SD*) (ml/min/1.73m^2)	
No History of Acute rejection	Time 1**	57.9 ± 34.6
	Time 2**	66.4 ± 33.7
History of Acute rejection	Time 1	84.5 ± 56.6
	Time 2	45.4 ± 25.8
Cadaveric donor	Time 1	53.7 ± 30.3
	Time 2	70.3 ± 36.8
Living donor	Time 1	88.8 ± 54.6
	Time 2	41.5 ± 21.6
Non-hypertension	Time 1	61.0 ± 38.1
	Time 2	66.2 ± 42.0
Hypertension	Time 1	81.5 ± 43.9
	Time 2	45.6 ± 27.1

* SD: standard deviation

** Time 1& Time 2: refers to the first and second serum creatinine measurement on record, respectively, regardless of whether it's the highest or lowest value on record.

Table 14: Multivariate, repeated measures, linear regression with GFR change over time as the dependent variable (Including the statistically significant medical variables and individual continuous periodontal variables):

Independent variables	F	P value	β	Partial Eta squared	Observed Power
History of acute rejection	4.80	.033	0.30	0.09	0.58
Living vs cadaveric donor	6.53	.014	0.34	0.12	0.71
Hypertension	4.01	.051	0.28	0.08	0.50
Percentage of sites with BOP	3.27	0.08	0.25	0.06	0.43
Mean PD	3.02	0.09	0.24	0.06	0.40
Percentage of sites with PD≥5mm	2.28	0.14	0.22	0.05	0.32

Table 15: Estimated means of first (older) and second (recent) GFR within the categories of each periodontal variable: percentage of sites with BOP, mean PD and percentage of sites with PD≥5mm:

Variable		GFR (Mean ± SD*) (ml/min/1.73m^2)	
Percentage of sites with BOP	(0% - <5%)	Time 1**	69.6 ± 37.9
		Time 2**	67.8 ± 46.4
	(5% – 25%)	Time 1	72.0 ± 47.2
		Time 2	45.5 ± 26.8
	(>25%)	Time 1	67.1 ± 38.9
		Time 2	55.0 ± 20.3
Percentage of sites with PD≥5mm	(0%)	Time 1	60.9 ± 35.0
		Time 2	60.2 ± 38.5
	(1 – 10%)	Time 1	75.1 ± 54.6
		Time 2	47.1 ± 26.7
	(>10%)	Time 1	77.1 ± 31.7
		Time 2	48.1 ± 24.8
Mean PD	(0.0 - 2.4)	Time 1	60.7 ± 38.8
		Time 2	64.2 ± 41.2
	(>2.4 - 3.1)	Time 1	71.5 ± 50.4

		Time 2	48.7 ± 29.4
	(>3.1)	Time 1	79.3 ± 32.2
		Time 2	45.8 ± 22.5

* SD: standard deviation

** Time 1& Time 2: refers to the first and second serum creatinine measurement on record, respectively, regardless of whether it's the highest or lowest value on record.

Table 16: Multivariate, repeated measures, linear regression with GFR change over time as the dependent variable (including the statistically significant medical variables and chronic periodontitis DEF2):

Independent variables	F	P value	β	Partial Eta squared	Observed Power
History of acute rejection	5.14	0.03	0.31	0.09	0.60
Living vs cadaveric donor	7.00	0.01	0.36	0.12	0.74
Hypertension	4.29	0.04	0.29	0.08	0.53
Chronic periodontitis DEF2	4.51	0.04	0.29	0.08	0.55

Table 17: Estimated means of first (older) and second (recent) GFR within the categories of Periodontitis DEF2 variable:

Variable		GFR (Mean ± SD*) (ml/min/1.73m^2)	
Chronic periodontitis (DEF2)	No periodontitis	Time 1**	59.1 ± 24.9
		Time 2**	77.9 ± 48.4
	periodontitis	Time 1	72.9 ± 44.7
		Time 2	46.8 ± 22.1

* SD: standard deviation

** Time 1& Time 2: refers to the first and second serum creatinine measurement on record, respectively, regardless of whether it's the highest or lowest value on record.

Table 18: Renal Biopsy Time Variables:

	Mean ±SD	Median (quartiles)
Time between transplant and biopsy (months)	66.1 ± 55.0	52.2 (21.4, 95.0)
Time between biopsy and most recent GFR (months)	-2.22 ± 11.6	0.07 (-0.14, 0.20)
Time between biopsy and oral exam (years)	1.9 ± 1.4	1.3 (0.8, 2.9)

Table 19: Frequency distribution of cadaveric, living related and living unrelated donors

Donor type	Frequency	Percentage
Cadaveric	32	56%
Living related	14	25%
Living unrelated	11	19%
Total	57	100%

Table 20: Cross-tabulation of donor type vs. history of acute rejection:

Donor type		History_of_acute_rejection		Total
		0	1	
Cadaveric	Frequency	16	13	29
	Percentage within donor type group	55.2%	44.8%	100.0%
	Percentage within history of acute rejection group	45.7%	68.4%	53.7%
	Percentage of Total	29.6%	24.1%	53.7%
Living	Frequency	19	6	25
	Percentage with donor type group	76.0%	24.0%	100.0%
	Percentage within history of acute rejection group	54.3%	31.6%	46.3%
	Percentage of Total	35.2%	11.1%	46.3%
Count		35	19	54
Percentage within donor type group		64.8%	35.2%	100.0%

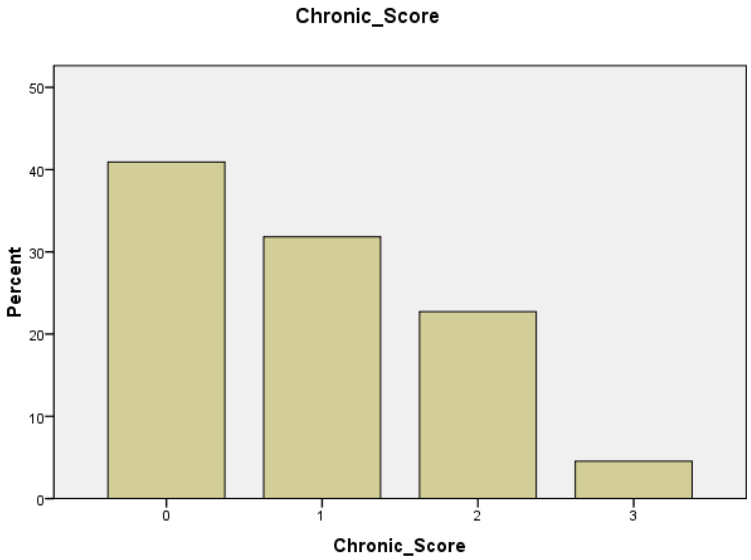
- Chi square (p=0.11)

Table 21: Cross-tabulation of donor type vs. number of HLA mismatches:

Donor type		Number of HLA mismatches		Total
		Less than 3	3 or more	
Cadaveric	Frequency	2	19	21
	Percentage within donor type group	9.5%	90.5%	100.0%
	Percentage within HLA mismatches group	40.0%	59.4%	56.8%
	Percentage of Total	5.4%	51.4%	56.8%
Living	Frequency	3	13	16
	Percentage with donor type group	18.8%	81.2%	100.0%
	Percentage within HLA mismatches group	60.0%	40.6%	43.2%
	Percentage of Total	8.1%	35.1%	43.2%
Count		35	5	32
Percentage within donor type group		64.8%	13.5%	86.5%

- Fisher's exact (p=0.42)

Figure 1: Percentage Distribution of CAN According to the Banff 97 Classification



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