ORAL DISEASES

Oral Diseases (2010) 16, 636–642. doi:10.1111/j.1601-0825.2010.01665.x

www.wiley.com

ORIGINAL ARTICLE

# Periodontitis case definition affects the association with renal function in kidney transplant recipients

**E Ioannidou[1]*, M Shaqman[1]*, J Burleson[2], A Dongari-Bagtzoglou[1]**

[1]*Division of Periodontology, Department of Oral Health and Diagnostic Sciences, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT, USA;* [2]*Department of Community Medicine and Health Care, School of Medicine, University of Connecticut Health Center, Farmington, CT, USA*

**AIM: The aim of this analysis was to investigate the association between periodontal status and renal allograft function in a cohort of renal transplant patients using different periodontitis case definitions.**

**MATERIAL AND METHODS: Fifty-eight kidney transplant patients were included. The subjects were classified into two groups, deterioration or stable/improvement of renal allograft function as expressed by the difference in glomerular filtration rate (GFR) between two time points at least 6 months apart. Chronic periodontitis was defined as: (1) two or more interproximal sites with clinical attachment level (CAL) ≥4 mm or two or more interproximal sites with probing depth (PD) ≥5 mm (DEF1); (2) PD ≥ 5 or CAL ≥ 4 in at least six proximal sites (DEF2); and (3) PD ≥ 5 or CAL ≥ 4 in at least two proximal sites in each quadrant (DEF3).**

**RESULTS: In a multivariate linear regression model, none of the continuous periodontal variables were significantly associated with deterioration of allograft function. Of the three definitions of chronic periodontitis, only DEF2 emerged as significantly more prevalent in subjects with GFR deterioration and was a statistically significant predictor of GFR deterioration over time.**

**CONCLUSION: These findings underscore the importance of periodontitis 'case definition' in the observed statistical associations between periodontitis and systemic disease.**

*Oral Diseases* (2010) **16**, 636–642

**Keywords:** chronic periodontitis; renal function; kidney transplant; glomerular filtration rate; periodontitis case definition; organ rejection

## Introduction

More than 16 500 kidney transplants are performed across the USA annually (Health Resources and Services Administration HS, 2007). Despite greatly improved 1-year survival rates of renal transplants from living and deceased donors (approximately 90–95%), the 10-year graft survival rates fall dramatically to 46–58% (Cecka, 2005). In renal transplants, 50–80% of these late failures are attributable to chronic allograft nephropathy (CAN) (Matas *et al*, 2001; Cecka, 2005), which is considered to be the most important cause of renal graft failure after the first year of transplantation (Dennis *et al*, 1989; Matas *et al*, 2001). Chronic allograft nephropathy 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 (Yates and Nicholson, 2006). Clinically, CAN presents as progressive deterioration in renal function (reduced glomerular filtration rate), proteinuria, and occasionally, *de novo* or secondary hypertension.

Several alloimmune-dependent factors [e.g. acute rejection episodes, human leukocyte antigen (HLA) mismatching] and alloimmune-independent factors (e.g. graft ischemia, brain death, obesity) are believed to influence renal graft function (Yates and Nicholson, 2006). Interestingly, a heightened pretransplant or post transplant systemic inflammation, as measured by a multitude of serum inflammation markers [e.g. C-reactive protein (CRP), vascular adhesion molecule-1, interleukin 12] has been associated with worse renal allograft outcomes (Perez *et al*, 2000, 2003; Fink *et al*, 2002; van Ree *et al*, 2007; Berber *et al*, 2008). Thus, it appears that elevated systemic inflammation sets the stage for an accentuated inflammatory response to the allograft and deterioration of graft function.

Systemic inflammation has been linked to periodontal inflammation. There is accumulating evidence that links periodontal disease to various systemic conditions, including cardiovascular disease (Bahekar *et al*, 2007), stroke (Desvarieux *et al*, 2003), diabetes mellitus (Taylor

Correspondence: Anna Dongari-Bagtzoglou, University of Connecticut Health Center, Division of Periodontology, 253 Farmington Ave, Farmington, CT 06030-1710, USA. Tel: 860-679-2363, Fax: 860-679-1027, E-mail: adongari@uchc.edu
*These authors contributed equally to this work.
Received 25 September 2009; revised 18 November 2009; accepted 2 December 2009

*et al*, 1996; Lamster and Lalla, 2001), poor pregnancy outcomes (Riche *et al*, 2002; Canakci *et al*, 2007; Ruma *et al*, 2008) and end-stage renal disease (Kshirsagar *et al*, 2009). One of the proposed mechanisms for this association is that these diseases share a common, genetically determined, susceptible phenotype whereby periodontitis and systemic disease are not causally related but both manifest in an individual with a hyper-inflammatory phenotype (Seymour *et al*, 2007). Several studies have shown a hyper-active phagocytic cell phenotype in chronic periodontitis patients (Fredriksson *et al*, 2003; Matthews *et al*, 2007), in which excessive production of inflammatory cytokines, proteases and reactive oxygen radicals result in destruction of periodontal tissues (Fredriksson *et al*, 2003; Matthews *et al*, 2007). Moreover, higher systemic inflammatory cytokine levels have been associated with periodontal disease (Takahashi *et al*, 1994; Noack *et al*, 2001; Ioannidou *et al*, 2006; Tonetti *et al*, 2007; Paraskevas *et al*, 2008). The latter observations also led to the hypothesis that periodontal inflammation may contribute incrementally to systemic inflammatory mediator levels and thereby amplify systemic disease processes (Seymour *et al*, 2007; Dave and Van Dyke, 2008).

Given the evidence that links periodontitis to a hyper-inflammatory phenotype (Fredriksson *et al*, 2003; Matthews *et al*, 2007) and higher systemic inflammatory cytokine levels in transplant (Ioannidou *et al*, 2006) and other patient populations (Noack *et al*, 2001; Nankivell *et al*, 2004; Paraskevas *et al*, 2008), we hypothesized that periodontal disease could serve as a marker to identify subjects with a systemic hyper-inflammatory phenotype that may be at greater risk for long-term renal allograft deterioration. The aim of this analysis was to investigate the association between the periodontal status of a cohort of renal transplant patients and renal allograft function.

## Materials and methods

*Subject recruitment*

The study that followed a cross-sectional single cohort design was approved by the University of Connecticut Health Center and Hartford Hospital Institutional Review Boards (IRB). The population under investigation was a subset of subjects from a larger investigation that included 90 renal and cardiac transplant patients. Renal transplant recipients were screened during routine outpatient visits at Hartford Hospital Transplant Center. Inclusion criteria were: (1) at least 1 year post transplant; (2) negative history of antibiotic use during the preceding 4 months; (3) no periodontal treatment within the last year; and (4) availability of at least two serum creatinine values 6 months apart and at least 6 months after the transplant. Fifty-eight renal transplant patients who met these criteria and signed the IRB approved informed consent forms were included.

*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), smoking status (current, former and never smoker), number of years post transplant, history of rejection episodes, medication regimen and dosage, graft rejection risk factors [e.g. HLA mismatching, panel reactive antibody (PRA) score, cadaveric or living donor, related or unrelated donor], creatinine clearance laboratory results and history of graft biopsy.

Using two serum creatinine values per subject as described in the inclusion criteria, two glomerular filtration rate (GFR) values (one at each time point) were calculated using the four-variable simplified Modification of Diet in Renal Disease (MDRD) formula [estimated GFR = $186 \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times (1.210 \text{ if Black}) \times (0.742 \text{ if Female})$] (Levey *et al*, 2006). Accordingly, the determination of renal allograft function over time was based on the difference:

$$\Delta\text{GFR} = \text{GFR}(\text{time1}) - \text{GFR}(\text{time2})$$

When $\Delta\text{GFR} > 0$, the subject was included in the deterioration group. Conversely, when $\Delta\text{GFR} \leq 0$, the subject was included in the stable/improvement group.

*Periodontal examination*

Subjects received a full-mouth periodontal examination at six sites per tooth. Four periodontists, who were calibrated prior to the initiation of the study, performed the exam using a Michigan O probe with Williams markings. The following parameters were evaluated and recorded: 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 GO index by Pernu *et al*, 1992. Chronic periodontitis was defined using three different definitions: the first definition (DEF1) was the Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP) working definition of moderate periodontitis. Accordingly, chronic periodontitis was defined as two or more interproximal sites with CAL ≥4 mm (not on the same tooth) or two or more interproximal sites with PD ≥5 mm (not on the same tooth). The second definition of chronic periodontitis (DEF2) was PD ≥ 5 or CAL ≥ 4 in at least six proximal sites. The third definition of chronic periodontitis (DEF3) was PD ≥ 5 or CAL ≥ 4 in at least two proximal sites in each quadrant. Both DEF2 and DEF3 were arbitrary definitions with clinical cutoff thresholds representing an escalating extent of periodontal destruction when compared with DEF1 (CDC/AAP definition).

*Statistical analyses*

A natural log transformation was applied to non-normally distributed variables. Mean and median were calculated for continuous variables. Student's *t*-test (for continuous variables) and chi-square test or Fisher's

exact test (for categorical variables) were used to test for differences between the groups (GFR deterioration *vs* stable/improvement). A multivariate, repeated measures, linear regression analysis was applied to determine significant predictors of the within-subject change in GFR values over time. Variables that turned a *P*-value $>0.2$ were excluded from the final regression model. A *P*-value $\leq 0.05$ was considered statistically significant. When $0.05 < P < 0.1$, we considered differences to have a trend to significance (Sterne and Smith, 2001).

## Results

The population demographic and medical data are reported in Tables 1 and 2 respectively. Diabetic patients comprised 52% of the subjects. There were no current smokers in this population. The median number of years post transplant was more than 7 years. Approximately, 60% of the subjects had a deterioration of GFR levels (mean $\Delta GFR = 56$ ml min$^{-1}$ 1.73 m$^{-2}$), whereas 40% of the subjects had an improvement of GFR levels (mean $\Delta GFR = -44$ ml min$^{-1}$ per 1.73 m$^{-2}$). Additional descriptive statistics on GFR values in the two groups appear in Table 3. Prevalence of chronic periodontitis according to case definition appears in Table 4, whereas specific periodontal parameters in the GFR stable/improvement *vs* deterioration groups are reported in Table 5. Although the prevalence of periodontitis was higher in the GFR deterioration group regardless of the case definition used, the difference was statistically significant only with DEF2 [$\chi^2$ (1, $n = 53$), $P = 0.04$, phi $= 0.28$] (Table 4). There were no statistically significant differences in mean: PD, CAL, percentage of sites with BOP, plaque score, percentage of sites with PD $\geq$5 mm, percentage of sites with CAL $\geq$ 4 mm or the number of missing teeth among the two groups (Table 5).

A multivariate, repeated measures, linear regression analysis was conducted to determine significant predictors of the within-subject GFR change over time (Table 6). The variables included were ethnicity, body mass index (BMI), diabetic status (yes/no), smoking history (former/never), living or cadaveric donor, hypertension, dialysis before transplantation, cold ischemic time, delayed graft function, acute rejection episodes (yes/no), PRA score, HLA mismatch (less than five *vs* five or more mismatches, prednisone use (yes/no), cyclosporine use (yes/no). Continuous periodontal parameters (mean PD, mean CAL, percentage of sites with BOP, percentage of sites with PD $\geq$ 5 mm, percentage of sites with CAL $\geq$ 4 mm) 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: gender, ethnicity, BMI, diabetes status (yes/no), smoking history (former/never), living or cadaveric donor, dialysis before transplantation, cold ischemic time, delayed graft function, PRA score, HLA mismatch (less than five *vs* five mismatches or more), prednisone use (yes/no), cyclosporine use (yes/no). History of acute rejection ($P = 0.03$), having a deceased donor ($P = 0.01$), and being hypertensive ($P = 0.05$) statistically significantly predicted deterioration of renal allograft function. When the continuous periodontal variables were introduced individually into the model,

**Table 1** Demographic characteristics

| *Variable* | *Mean ± s.d. (median)* |
|---|---|
| Age | 52.8 ± 12.0 (53.3) |
| Gender (female) (%) | 41 |
| Ethnicity (Black) (%) | 24 |

**Table 2** Medical characteristics

| *Variable* | *Mean, median (quartiles)* |
|---|---|
| Diabetics (%) | 52 |
| Smoking (former) (%) | 31 |
| Years post transplant | 7.6 ± 4.9, 7.1 (3.8, 11.0) |
| History of acute rejection (%) | 33 |
| Pretransplant dialysis (%) | 78 |
| Living donor (related or unrelated) (%) | 43 |
| Ca++ channel blockers (%) | 28 |
| Cyclosporine (%) | 38 |
| Prednisone (%) | 91 |
| Mycophenolate mofetil (%) | 72 |
| Tacrolimus (%) | 57 |
| Azathioprine (%) | 21 |
| Sirolimus (%) | 14 |

**Table 3** Mean, median and quartiles of ΔGFR values in the deterioration and stable/improvement groups

| *Variable* | *Mean, median (quartiles)* |
|---|---|
| Mean ΔGFR in deterioration group (ml min$^{-1}$ per 1.73 m$^2$) | 56, 48 (66,36) |
| Mean ΔGFR in stable/improvement group (ml min$^{-1}$ per 1.73 m$^2$) | −44, −44 (−26, −56) |

GFR, glomerular filtration rate.

**Table 4** Chronic periodontitis (CP) frequency (subject numbers and percentages) in each glomerular filtration rate (GFR) group according to the three definitions

| *Definition* | *GFR deterioration (%)* | *GFR stable/improvement (%)* | *Total in both GFR groups (%)* | P-*value* |
|---|---|---|---|---|
| DEF1 | 25 (71) | 13 (57) | 38 (65.5) | 0.37* (NS) |
| DEF2 | 32 (91) | 16 (70) | 48 (82.8) | 0.04* |
| DEF3 | 20 (57) | 9 (39) | 29 (50) | 0.18** (NS) |

GFR, glomerular filtration rate.
*$\chi^2$ test.
**Fisher's exact test.

**Table 5** Continuous periodontal variables in the two glomerular filtration rate (GFR) groups

| | *GFR deterioration* | | *GFR improvement* | | |
|---|---|---|---|---|---|
| *Variable* | *Mean ± s.d.* | *Median (25th,75th quartiles)* | *Mean ± s.d.* | *Median (25th,75th quartiles)* | P-*value** |
| 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 (%) | 18.8 ± 15.5 | 15.0 (6.5, 25.0) | 14.7 ± 13.8 | 7.7 (4.6, 28.5) | 0.14 (NS) |
| PS (%) | 50.7 ± 29.0 | 53.6 (28.3, 76.9) | 43.0 ± 33.0 | 42.4 (10.7, 74.4) | 0.35 (NS) |
| 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 ≥ 4 mm (%) | 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 ≥ 5 mm (%) | 7.0 ± 9.2 | 2.4 (0.0, 12.3) | 5.6 ± 9.0 | 0.0 (0.0, 11.4) | 0.22 (NS) |

PS, plaque score; PD, probing depth; GFR, glomerular filtration rate; BOP, bleeding on probing.

**Table 6** Multivariate, repeated measures, linear regression models designed based on the three chronic periodontitis 'case definitions'

| *Variable* | *F* | *Sig.* | *β* | *Observed power* |
|---|---|---|---|---|
| Model 1 | | | | |
| History of acute rejection | 7.02 | 0.01 | 0.36 | 0.57 |
| Cadaveric donor | 6.66 | 0.01 | 0.35 | 0.54 |
| Hypertension | 4.34 | 0.04 | 0.29 | 0.80 |
| Chronic periodontitis (DEF1) | 0.05 | 0.83 | 0.03 | 0.06 |
| Model 2 | | | | |
| History of acute rejection | 5.14 | 0.03 | 0.31 | 0.60 |
| Cadaveric donor | 7.00 | 0.01 | 0.36 | 0.74 |
| Hypertension | 4.29 | 0.04 | 0.29 | 0.53 |
| Chronic periodontitis (DEF2) | 4.51 | 0.04 | 0.29 | 0.55 |
| Model 3 | | | | |
| History of acute rejection | 4.79 | 0.03 | 0.30 | 0.57 |
| Cadaveric donor | 6.52 | 0.01 | 0.34 | 0.71 |
| Hypertension | 4.00 | 0.05 | 0.28 | 0.50 |
| Chronic periodontitis (DEF3) | 0.89 | 0.35 | 0.13 | 0.15 |

higher percentage of sites with BOP and higher mean PD showed a trend towards statistical significance in predicting deterioration of allograft function (percentage sites with BOP: $F = 3.27$, $P = 0.08$; mean PD: $F = 3.02$, $P = 0.09$). Other continuous periodontal variables were not statistically significant in the model (percentage of sites with PD ≥5 mm: $F = 2.28$, $P = 0.14$; mean CAL: $F = 0.06$, $P = 0.81$, percentage of sites with CAL ≥4 mm: $F = 0.74$, $P = 0.39$; number of missing teeth: $F = 0.15$, $P = 0.70$). Chronic periodontitis as defined by DEF2 emerged as a statistically significant predictor of GFR deterioration over time. In contrast, chronic periodontitis according to DEF1or DEF3 was not significant a predictor (Table 6).

## Discussion

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 (Meier-Kriesche *et al*, 2004). Several parameters have been shown to be correlated with long-term allograft outcomes such as acute rejection episodes (Matas *et al*, 1994), HLA mismatches (McKenna *et al*, 1998), delayed graft function (Shoskes and Cecka, 1997), and having a living *vs* a cadaveric donor (Massy *et al*, 1996). Nevertheless, these parameters can not explain the variation in the clinical course and outcome of organ transplantation.

In this study, we hypothesized that periodontal disease is one of the markers of a systemic hyperinflammatory phenotype and if present in renal transplant recipients, it might indicate a greater risk for long-term renal allograft deterioration. Although this hypothesis does not necessarily suggest causality, this association would have several implications in this patient population. For example, a history of periodontitis would 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 surveillance biopsies might be indicated. Conventionally, graft failure is suspected only when a continued and irreversible reduction of renal function has become clinically apparent, as a combination of hypertension and proteinuria, which prompts performance of renal allograft biopsies when it may already be too late (Morath *et al*, 2003).

This analysis was performed on a subset of the renal allograft recipients from a larger renal and cardiac transplant recipient population. One of the inclusion criteria was the availability of two serum creatinine lab results that were taken at least 6 months after the transplantation and were at least 6 months apart. Those cutoff points were chosen as one large study had shown that allograft function at 6 months post transplant, as measured by creatinine values, and the changes that occurred 6 months later were related to the 5-year survival of the graft (Hariharan *et al*, 2002). 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 when compared with the Cockcroft & Gault formula (Cockcroft and Gault, 1976; Poge *et al*, 2005). The mean ΔGFR values and frequency of improvement/deterioration over time in our population are in agreement with longitudinal observations of similar populations in other studies (Wigger *et al*, 2001; Gera *et al*, 2007). More importantly, all subjects with a history of renal biopsy

were found to be in the deterioration group, which was on expected lines, as renal biopsy is indicated only when renal function is reduced (Morath *et al*, 2003).

Traditionally, investigators have used arbitrary definitions of chronic periodontitis in epidemiologic studies assessing the relationship with systemic diseases (Tonetti *et al*, 2005; Manau *et al*, 2008, de Pablo *et al*, 2008). In this analysis, periodontal destruction was measured using a series of continuous variables and three different 'disease case' definitions. The decision to include more than one case definition was triggered by a recent study which showed that when different disease definitions were applied in the same patient population, considerably different associations were found between periodontitis and systemic diseases (Manau *et al*, 2008). In agreement with this study, we found that only one of the three case definitions provided statistically significant results in a multivariate regression model. Other medical variables (history of rejection, cadaveric donor and hypertension) found to be significant are in agreement with previous studies (Matas *et al*, 1994, 2001; Nickerson *et al*, 1998). Although an association between periodontitis, human cytomegalovirus (HCMV) infection and renal transplant complications has been found (Nowzari *et al*, 2003), we were unable to confirm this because of non-availability of HCMV data in our population.

Continuous periodontal variables were not significantly different between the GFR groups. However, clinically small trends were observed for greater periodontal destruction in the GFR deterioration group. In addition, using all definitions, chronic periodontitis showed higher prevalence in the GFR deterioration group when compared with the GFR improvement group. These findings suggest that periodontal destruction might be a risk indicator for renal allograft deterioration. Furthermore, when continuous periodontal variables were tested in the multivariate regression model, there was a suggestion that the extent and severity of periodontal destruction, as expressed by percentage of sites with BOP and/or mean PD, could predict GFR deterioration. Thus, the 'periodontitis' case definitions tested in this study attempted to capture an escalating severity of the disease, from DEF1 (lowest) to DEF3 (highest) severity. Surprisingly, only the case definition representing a 'medium size' disease severity (DEF2) showed statistically significant findings. This finding would argue against a strictly linear relationship between disease severity and the observed associations. However, the cutoff points used in DEF2 were more strict in defining disease severity when compared with other clinical studies that used definitions with either fewer periodontally involved sites (Bassani *et al* 2007, Holbroook *et al* 2004, Jarjoura *et al* 2005, Jeffcoat *et al* 2001) or lower PD or CAL cutoff points (Goepfert *et al* 2004, Holbroook *et al* 2004, Jarjoura *et al* 2005, Jeffcoat *et al* 2001).

Our *a priori* power analysis to investigate differences in the prevalence of chronic periodontitis in kidney or heart transplant subjects when compared with systemically healthy controls, revealed the need for a minimum of 54 subjects, for a power of 80% and alpha-value of 0.05. In this subgroup analysis of 58 kidney transplant subjects, we applied three different definitions that had not been considered in our *a priori* power calculations. *Post hoc* power calculations revealed that in order to attain this level of power for each case definition in a multivariate regression model with four predictors (as shown in Table 6), we needed a minimum of 96 subjects for DEF 1, 48 subjects for DEF2, and 71 subjects for DEF3. This may raise the question whether an adequate number of subjects were available for generating statistically significant comparisons using DEF1 or DEF3. However, although such *post hoc* power analyses are tempting, they are not conclusive and often lead to misinterpretation of the results. For example, even for a study with *a priori* sufficient power, if the results are negative, the *post hoc* power analysis based on the observed results will always show that the study was underpowered (Levine and Ensom, 2001, Freedman, 1999).

An important limitation of our analysis was that the most recent serum creatinine test was not performed at the time of the oral examination but was retrieved from the medical record. However, the major focus of this investigation was to test the effect of case definition selection on the observed associations between renal function and periodontitis within a kidney transplant population. Moreover, the findings of this study could contribute to power estimates of future longitudinal studies with prospective monitoring of allograft function, periodontal status and protocol biopsies in a larger population that will provide definitive conclusions on the association between periodontitis and renal function in this and other populations.

In conclusion, our findings suggest that chronic periodontitis is more prevalent in the GFR deterioration group and may be a predictor for GFR deterioration over time. However, the impact of the disease thresholds that were used to define a 'periodontitis case' was also evident in these associations. These findings underscore the importance of the 'case definition of periodontitis' selection in studies investigating the link between periodontitis and systemic diseases. Based on these findings, we propose that future studies in this field use more than one case definition to strengthen the validity of the observed associations.

## Acknowledgements

This study was supported by NIH/NIDCR Research grants R21DE16466 awarded to AD-B and K23DE018689 awarded to EI. This research was also supported in part by a General Clinical Research Center grant from NIH (M01RR06192) awarded to UCHC. Special thanks to Dr. David Hull, Head of the Transplant Unit – Hartford Hospital, for his contribution.

## Conflict of interest

This study was supported by the National Institute of Health, National Institute of Dental and Craniofacial Research grants awarded to ADB (R21DE16466) and to EI (K23DE018689).

This research was also supported in part by a General Clinical Research Center grant from NIH (M01RR06192) awarded to UCHC.

## References

Bahekar AA, Singh S, Saha S, Molnar J, Arora R (2007). The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am Heart J* **154:** 830–837.

Bassani DG, Olinto MT, Kreiger N (2007). Periodontal disease and perinatal outcomes: a case-control study. *J Clin Periodontol* **34:** 31–39.

Berber I, Yigit B, Isitmangil G *et al* (2008). Evaluation of pretransplant serum cytokine levels in renal transplant recipients. *Transplant Proc* **40:** 92–93.

Canakci V, Canakci CF, Yildirim A, Ingec M, Eltas A, Erturk A (2007). Periodontal disease increases the risk of severe pre-eclampsia among pregnant women. *J Clin Periodontol* **34:** 639–645.

Cecka JM (2005). The OPTN/UNOS Renal Transplant Registry. *Clin Transpl*, 1–16.

Cockcroft DW, Gault MH (1976). Prediction of creatinine clearance from serum creatinine. *Nephron* **16:** 31–41.

Dave S, Van Dyke T (2008). The link between periodontal disease and cardiovascular disease is probably inflammation. *Oral Dis* **14:** 95–101.

Dennis MJ, Foster MC, Ryan JJ, Burden RP, Morgan AG, Blamey RW (1989). The increasing importance of chronic rejection as a cause of renal allograft failure. *Transpl Int* **2:** 214–217.

Desvarieux M, Demmer RT, Rundek T *et al* (2003). Relationship between periodontal disease, tooth loss, and carotid artery plaque: The Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Stroke* **34:** 2120–2125.

Fink JC, Onuigbo MA, Blahut SA *et al* (2002). Pretransplant serum C-reactive protein and the risk of chronic allograft nephropathy in renal transplant recipients: a pilot case-control study. *Am J Kidney Dis* **39:** 1096–1101.

Fredriksson MI, Gustafsson AK, Bergstrom KG, Asman BE (2003). Constitutionally hyperreactive neutrophils in periodontitis. *J Periodontol* **74:** 219–224.

Freedman KB, Bernstein J (1999). Sample size and statistical power in clinical orthopaedic research. *J Bone Joint Surg Am* **81:** 1454–1460.

Gera M, Slezak JM, Rule AD, Larson TS, Stegall MD, Cosio FG (2007). Assessment of changes in kidney allograft function using creatinine-based estimates of glomerular filtration rate. *Am J Transplant* **7:** 880–887.

Goepfert AR, Jeffcoat MK, Andrews WW, *et al*. (2004). Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. *Obstet Gynecol* **104:** 777–783.

Hariharan S, McBride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP (2002). Post-transplant renal function in the first year predicts long-term kidney transplant survival. *Kidney Int* **62:** 311–318.

Health Resources and Services Administration HS (2007). 2007 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1997–2006. Health Resources and Services Administration HS. Available at: www.ustransplant.org; http://www.ustransplant.org (accessed 8 January 2008).

Holbrook WP, Oskarsdottir A, Fridjonsson T, Einarsson H, Hauksson A, Geirsson RT (2004). No link between low-grade periodontal disease and preterm birth: a pilot study in a healthy Caucasian population. *Acta Odontol Scand* **62:** 177–179.

Ioannidou E, Malekzadeh T, Dongari-Bagtzoglou A (2006). Effect of periodontal treatment on serum C-reactive protein levels: a systematic review and meta-analysis. *J Periodontol* **77:** 1635–1642.

Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'Alton M, Papapanou PN (2005). Markers of periodontal infection and preterm birth. *Am J Obstet Gynecol* **192:** 513–519.

Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC (2001). Periodontal infection and preterm birth: results of a prospective study. *J Am Dent Assoc* **132:** 875–880.

Kshirsagar AV, Craig RG, Moss KL *et al* (2009). Periodontal disease adversely affects the survival of patients with end-stage renal disease. *Kidney Int* **75:** 746–751.

Lamster IB, Lalla E (2001). Periodontal disease and diabetes mellitus: discussion, conclusions, and recommendations. *Ann Periodontol* **6:** 146–149.

Levey AS, Coresh J, Greene T *et al* (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* **145:** 247–254.

Levine M, Ensom M (2001). *Post hoc* power analysis: an idea whose time has passed? *Pharmacotherapy* **21:** 405–409.

Manau C, Echeverria A, Agueda A, Guerrero A, Echeverria JJ (2008). Periodontal disease definition may determine the association between periodontitis and pregnancy outcomes. *J Clin Periodontol* **35:** 385–397.

Massy ZA, Guijarro C, Wiederkehr MR, Ma JZ, Kasiske BL (1996). Chronic renal allograft rejection: immunologic and nonimmunologic risk factors. *Kidney Int* **49:** 518–524.

Matas AJ, Gillingham KJ, Payne WD, Najarian JS (1994). The impact of an acute rejection episode on long-term renal allograft survival (t1/2). *Transplantation* **57:** 857–859.

Matas AJ, Payne WD, Sutherland DE *et al* (2001). 2,500 living donor kidney transplants: a single-center experience. *Ann Surg* **234:** 149–164.

Matthews JB, Wright HJ, Roberts A, Ling-Mountford N, Cooper PR, Chapple IL (2007). Neutrophil hyper-responsiveness in periodontitis. *J Dent Res* **86:** 718–722.

McKenna RM, Lee KR, Gough JC *et al* (1998). Matching for private or public HLA epitopes reduces acute rejection episodes and improves two-year renal allograft function. *Transplantation* **66:** 38–43.

Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B (2004). Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant* **4:** 378–383.

Morath C, Ritz E, Zeier M (2003). Protocol biopsy: what is the rationale and what is the evidence? *Nephrol Dial Transplant* **18:** 644–647.

Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD (2004). Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* **78:** 557–565.

Nickerson P, Jeffery J, Gough J *et al* (1998). Identification of clinical and histopathologic risk factors for diminished renal function 2 years posttransplant. *J Am Soc Nephrol* **9:** 482–487.

Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E (2001). Periodontal infections contribute to elevated systemic C-reactive protein level. *J Periodontol* **72:** 1221–1227.

Nowzari H, Jorgensen MG, Aswad S *et al* (2003). Human cytomegalovirus-associated periodontitis in renal transplant patients. *Transplant Proc* **35:** 2949–2952.

de Pablo P, Dietrich T, McAlindon TE (2008). Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *J Rheumatol* **35:** 70–76.

Paraskevas S, Huizinga JD, Loos BG (2008). A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* **35:** 277–290.

Perez RV, Brown DJ, Katznelson SA *et al* (2000). Pretransplant systemic inflammation and acute rejection after renal transplantation. *Transplantation* **69:** 869–874.

Perez RV, Huang CQ, Johnson JR *et al* (2003). Pretransplantation soluble adhesion molecule expression predicts outcome after living donor renal transplantation. *Arch Surg* **138:** 1113–1119.

Pernu HE, Pernu LM, Huttunen KR, Nieminen PA, Knuuttila ML (1992). Gingival overgrowth among renal transplant recipients related to immunosuppressive medication and possible local background factors. *J Periodontol* **63:** 548–553.

Poge U, Gerhardt T, Palmedo H, Klehr HU, Sauerbruch T, Woitas RP (2005). MDRD equations for estimation of GFR in renal transplant recipients. *Am J Transplant* **5:** 1306–1311.

van Ree RM, Oterdoom LH, de Vries AP *et al* (2007). Elevated levels of C-reactive protein independently predict accelerated deterioration of graft function in renal transplant recipients. *Nephrol Dial Transplant* **22:** 246–253.

Riche EL, Boggess KA, Lieff S *et al* (2002). Periodontal disease increases the risk of preterm delivery among preeclamptic women. *Ann Periodontol* **7:** 95–101.

Ruma M, Boggess K, Moss K *et al* (2008). Maternal periodontal disease, systemic inflammation, and risk for preeclampsia. *Am J Obstet Gynecol* **198:** 389.e1–e5.

Seymour GJ, Ford PJ, Cullinan MP, Leishman S, Yamazaki K (2007). Relationship between periodontal infections and systemic disease. *Clin Microbiol Infect* **13** (Suppl. 4)**:** 3–10.

Shoskes DA, Cecka JM (1997). Effect of delayed graft function on short- and long-term kidney graft survival. *Clin Transpl*, 297–303.

Sterne J, Smith GD (2001). Sifting the evidence-what's wrong with significance tests? *BMJ* **322:** 226–230.

Takahashi K, Takashiba S, Nagai A *et al* (1994). Assessment of interleukin-6 in the pathogenesis of periodontal disease. *J Periodontol* **65:** 147–153.

Taylor GW, Burt BA, Becker MP *et al* (1996). Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *J Periodontol* **67:** 1085–1093.

Tonetti MS, Claffey N, European Workshop in Periodontology group C (2005). Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. *J Clin Periodontol* **32:** 210–213.

Tonetti MS, D'Aiuto F, Nibali L *et al* (2007). Treatment of periodontitis and endothelial function. *N Engl J Med* **356:** 911–920.

Wigger M, Druckler E, Muscheites J, Stolpe HJ (2001). Course of glomerular filtration rate after renal transplantation and the influence of hypertension. *Clin Nephrol* **56:** S30–S34.

Yates PJ, Nicholson ML (2006). The aetiology and pathogenesis of chronic allograft nephropathy. *Transpl Immunol* **16:** 148–157.