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**Abstract.** The Z-Scan (ZS) technique in the thermal regime has been used to measure the nonlinear optical response of low-density lipoprotein (LDL). The ZS technique is carried out in LDL from 40 patients with chronic periodontitis before and after three, six, and 12 months of periodontal treatment. Clinical parameters such as probing depths, bleeding on probing, total and differential white blood cells counts, lipid profiles, cytokine levels, and antibodies against oxidized LDL are also determined and compared over time. Before the treatment, the ZS experimental results reveal that the LDL particles of these patients are heavily modified. Only after 12 months of the periodontal treatment, the ZS results obtained reveal behavioral characteristics of healthy particles. This conclusion is also supported by complementary laboratorial analysis showing that the periodontal treatment induces systemic changes in several inflammatory markers. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: [10.1117/1.JBO.17.11.115004](https://doi.org/10.1117/1.JBO.17.11.115004)]

Keywords: Z-scan; nonlinear; atherosclerosis; inflammation; oral health; oxLDL; periodontitis.

Paper 12462 received Jul. 18, 2012; revised manuscript received Oct. 19, 2012; accepted for publication Oct. 22, 2012; published online Nov. 8, 2012.

## 1 Introduction

Periodontitis, an inflammatory disease caused by gram-negative bacteria, is characterized by alveolar bone destruction and, in some cases, tooth loss. During the last two decades, several studies have demonstrated that periodontitis is an important contributing factor in the pathogenesis of systemic diseases,<sup>1</sup> particularly cardiovascular disease.<sup>2,3</sup> The contribution of periodontitis to the atherosclerosis process is shown by the increased serum levels of several inflammatory markers, including cytokines, which are induced directly by an interaction between host cells and periodontal pathogens.<sup>4</sup> High levels of inflammatory cytokines present in the inflamed periodontal tissue can access the blood circulation and contribute to the atherosclerotic process by altering lipid metabolism.<sup>5</sup> Inflammatory reactions and increased levels of plasma lipoproteins, e.g., triacylglycerol (TG) and low-density lipoprotein (LDL), are well known for their pro-atherogenic properties. Moreover, the generation of reactive oxygenic species (ROS), an important mechanism for the elimination of microbial pathogens,<sup>6</sup> can cause oxidative modifications of LDL (oxLDL) and thus contribute to increased atherosclerotic risk. Bengtsson et al.<sup>7</sup> showed that oral bacteria can cause modifications and degradation of LDL particles.

Moreover, it was demonstrated that the oxLDL level in gingival crevicular fluid is, on average, 17 times greater than the plasma oxLDL levels in the same subjects, providing evidence that the oxidative modifications of LDL can occur in the mouth.<sup>8</sup> OxLDL induces an immune response in which antibodies or T-cells against oxLDL can be detected.<sup>9,10</sup> Immune response against oxLDL is in the heterogeneous group and can be used as an indirect marker for the presence of oxLDL in circulation.<sup>11</sup> Most interestingly, studies have demonstrated an association between oxLDL Ab titer and the progression of atherosclerosis. Thus oxLDL has been suggested as a marker for the progression of atherosclerosis.<sup>12,13</sup>

Although several population studies have established an association between oral health and cardiovascular risk,<sup>14–16</sup> few have analyzed the effect of periodontal treatment on cardiovascular risk, leaving some questions unanswered.<sup>17,18</sup> This is particularly true due to certain variables, including different population samples, the severity of periodontal disease, the type of periodontal treatment provided, the duration of follow-up, and the markers used. In the majority of cases, patients are analyzed after three and/or six months and investigated for the consequences of the single nonsurgical debridement of periodontal treatment. In this study, patients were examined before and during the 12-month period after periodontal treatment consisting of extraction and surgery. The markers studied were

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acute and chronic cytokine markers, high-sensitivity C-reactive protein (hsCRP), and lipid and hematological profiles.

Some years ago, our research group showed that the nonlinear optical responses of native and modified LDL solutions, measured with the Z-Scan technique<sup>19</sup> in the thermal regime, are different.<sup>20</sup> This tool allows an objective assessment of the LDL's degree of oxidation in the patient's blood through the amplitude of the Z-scan response. To the best of our knowledge, there is no study in the literature that investigates over 12 months the effect of periodontal treatment on the atherogenic level of LDL and the presence of anti-oxLDL antibodies in patients with periodontitis under treatment. A study of this type also highlights several issues:

- (1) whether markers commonly used in clinical interventions on patients with coronary artery disease (CAD) behave in individuals who underwent only periodontal treatment
- (2) whether the treatment and changes seen are permanent and synchronized
- (3) whether periodontal treatment causes alterations in the levels of anti-mLDL antibodies and the oxidative status of LDL.

## 2 Materials and Methods

### 2.1 Patient Selection

This study was a prospective and intervention pilot trial with a 12-month followup. Subjects were recruited at the Department of Periodontology of the São Paulo Dental Association, São José dos Campos, SP, Brazil. Individuals were excluded if they had a history of cardiovascular disease, diabetes mellitus, hypertension, or smoking. Individuals with any systemic disease or who had been treated for periodontitis within six months of the present study were also excluded. None of the individuals investigated took cardiovascular or anti-inflammatory medication or antibiotics within the three months prior to enrollment in the study. Furthermore, individuals with pulpar or periapical infections were excluded. Inclusion criteria for subjects with chronic periodontitis were  $\geq 16$  teeth and  $\geq 10$  sites with a probing depth (PD) of 5 mm.

A total of 40 patients with chronic periodontitis were enrolled in this study according to the previously described criteria, with 22 (55%) females and 18 (45%) males. Their mean age was  $45.2 \pm 9.9$  years. This study was approved by the Medical Ethical Committee for Human Beings of the Institute of Biomedical Sciences, University of São Paulo. All participants were informed about the procedure and provided written consent.

### 2.2 Clinical Examination

All clinical parameters were assessed by a single periodontitis expert, and a calibration exercise was performed to obtain acceptable intra-examiner reproducibility. The evaluation included bleeding on probing (BOP) as an indication of existing periodontal inflammation. PD and gingival recession were recorded for six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual), with the exception of the third molar, using a pressure-calibrated

digital recording device (Florida Probe, Gainesville, FL). A fasting blood sample was taken after the periodontal examination but before the treatment began. Each individual was codified and randomized, and additional analysis was performed without previous knowledge of the state of the individual from whom the sample was drawn.

### 2.3 Clinical Treatment of Periodontal Disease

All patients received oral hygiene instructions. Individuals underwent a full-mouth intensive-treatment removal of supra- and subgingival dental plaque and calculus by scaling and root planning. The extraction of hopeless teeth due to periodontitis was also performed. Those patients who still had PD > 5 mm after the nonsurgical treatment then underwent standardized periodontal muco-periosteal flap surgery. After the surgical treatment, the patients were re-examined. Clinical parameters were made at three, six, and 12 months.

### 2.4 Laboratory Analyses

Blood was collected into Vacutainer tubes containing EDTA from each individual after 12 h of fasting at baseline and three, six, and 12 months. The hematological parameters were measured in standard automated setups. Plasma was obtained after centrifugation of the blood at 1000 g for 10 min at 4°C and stored at -70°C. Total cholesterol (TC), TG, and high-density lipoprotein (HDL) were quantified by enzymatic methods (Lab-test, Lagoa Santa, Minas Gerais, Brazil). LDL was calculated using the Friedewald formula.<sup>21</sup> Highly purified LDL from the plasma was extracted by sequential ultracentrifugation<sup>22</sup> at 10<sup>5</sup> g at 4°C using the P50AT4 rotor of an ultracentrifuge (Hitachi Ultracentrifuge, Tokyo, Japan) and dialyzed at 4°C against phosphate buffer saline (PBS – pH = 7.4) with 0.01% EDTA. Protein concentration was determined by a BCA kit (Pierce, USA) using BSA as the standard. The levels of plasma anti-oxLDL antibodies were determined as described previously.<sup>11</sup> Hs-CRP concentrations were determined by nephelometry (R100 Analyser, Behringer, Germany). Interleukin (IL)-1 $\beta$ , -6, -8 and -10 and tumor necrosis factor (TNF)- $\alpha$  concentrations were determined using commercially available quantitative ELISA (Becton Dickinson, San Jose, CA).

### 2.5 Z-Scan (ZS) in the Milliseconds Time-Scale Regime

In this time-scale, the ZS experiment is sensitive mainly to the radial temperature gradient in the sample. The laser light absorbed by the sample is converted into heat. As the sample refractive index  $n$  depends, among other factors, on temperature, an increment  $\Delta T$  in temperature induces a change  $\Delta n = (dn/dT)\Delta T$  in the refraction index.<sup>23</sup> The parameter  $dn/dT$  is the thermo-optic coefficient. In the present work, a laser beam with a Gaussian-shaped intensity profile (mode TEM<sub>00</sub>) induces a thermal lens in the medium, which depends on the medium properties, particularly the thermo-optic coefficient and the thermal diffusivity  $D$ . In the ZS technique, a polarized Gaussian beam is focused to a narrow waist by a lens, and a sample is moved through the focal point. The transmittance  $\Gamma$  of an iris, centered in the  $z$ -axis (propagating axis of the laser beam) in the far field, is measured as a function of the position of the sample along the  $z$ -axis. In the parabolic approximation of the induced thermal lens,<sup>24</sup> the amplitude of the Z-scan signal,

i.e., the difference in transmittance from peak-to-valley  $\Delta\Gamma_{p-v}$  in the stationary state, is proportional<sup>24</sup> to the dimensionless parameter  $\theta$ . The normalized transmittance at the iris is written as

$$\frac{\Gamma(t, z)}{\Gamma(0, z)} = \left\{ 1 + \frac{\theta}{(1 + t_c/2t)} \left[ \frac{2(z/z_0)}{1 + (z/z_0)^2} \right] + \left[ \frac{\theta}{(1 + t_c/2t)} \right]^2 \left[ \frac{1}{1 + (z/z_0)^2} \right] \right\}^{-1}, \quad (1)$$

where  $z_0$  is the Rayleigh length ( $z_0 = \pi\omega_0^2/\lambda$ ),  $\lambda$  is the laser wavelength, and  $\omega_0$  is the spot radius at the beam waist,

$$\theta = \frac{2.303PA}{\lambda D \rho c_p} \left( -\frac{dn}{dT} \right), \quad (2)$$

and

$$t_c = \frac{\omega_0^2[1 + (z/z_0)^2]}{4D}, \quad (3)$$

where  $P$  is the beam power in the sample,  $A = -\log 10(1 - \alpha L)$  is the decadic absorbance,  $\alpha$  is the linear optical absorption coefficient at  $\lambda$ ,  $L$  is the sample thickness,  $c_p$  is the heat capacity at constant pressure, and  $D$  is the thermal diffusivity.

The Z-scan apparatus used here is the usual one described elsewhere.<sup>20</sup> Our setup uses a cw laser (Verdi V10, Coherent) of wavelength  $\lambda = 532$  nm. A mechanical chopper modulates the laser beam incident on the sample, providing nearly square pulses of  $\sim 30$  ms (i.e., 30 ms in the “on” state, with the laser lighting the sample, and 30 ms in the “off” state). The beam waist of the laser at focus  $\omega_0$  was about  $24.2 \pm 0.1$   $\mu\text{m}$ , the Rayleigh length of Gaussian beam was  $z_0 = 3.63 \pm 0.9$  mm, and the power of the laser was  $254 \pm 3$  mW. Samples were encapsulated in tightly sealed rectangular glass cells (thickness 200  $\mu\text{m}$ ). For a more detailed description of the application of the ZS technique to lipoprotein biological systems, see Gomes et al.<sup>20</sup> All samples were blinded and analyzed at the same time under the same conditions.

## 2.6 Statistical Analysis

Mixed regression linear models were used to establish whether there were any treatment effects over time (three time points) on the levels of plasma cytokines and systemic inflammation markers, with separate models for each variable. Diagnostic analyses were performed to check the adequacy of the model and identify possible outliers. Where the assumption that normality was not satisfied, the data were transformed to normality with the appropriate power transformation of Box-Cox.

Initially, multivariate models that included possible factors confounding the treatment effect, such as sex, body mass index (BMI), and age, were fitted, but none of these factors were statistically significant according to the F-test. Therefore, the final models included time as the random effect factor and patient visits. Post hoc comparisons between visits were undertaken if the F-test was significant.

Significant differences between periodontal parameters before and after treatment were calculated using the Mann-Whitney U-test.

All statistical analyses were performed using the Language and Environment for Statistical Computing R version 2.13.2.

## 3 Results and Discussion

We previously reported the presence of a number of inflammatory mediators as possible factors that link periodontal disease to elevated cardiovascular risk in patients with chronic periodontitis compared with individuals without periodontal disease.<sup>25</sup> In the present study, the influence of periodontal treatment on the serum levels of inflammatory biomarkers for CAD was investigated. All 40 patients who were initially enrolled in this study completed the 12-month followup. Furthermore, these patients did not have any clinical symptoms of bacterial, viral, or parasitic infections during the study. The baseline characteristics of the individuals are summarized in Table 1. Removal of the infection sites successfully reduced the intensity of local infection and inflammation. The clinical markers of disease decreased with treatment and remained stable until 12 months, as observed in the BOP and the amount of PD > 5 mm (Table 1). Possible correlations between clinical status and plasma variables were tested at baseline and at three, six, and 12 months after periodontal treatment. None of the investigated plasma variables were correlated with the clinical status ( $p > 0.05$ , data not shown).

When TC ( $p$ -value = 0.74), HDL ( $p$ -value = 0.92), and LDL ( $p$ -value = 0.94) levels were compared, no significant differences were observed between levels before and after treatment (Table 2). TG levels were not statistically significant before or three months after treatment ( $p$ -value = 0.32). Six months after periodontal treatment, however, TG concentrations decreased significantly when compared with those before treatment ( $p$ -value = 0.03), as shown in Table 2. Our results indicate that periodontal treatment influences plasma lipid levels, resulting in a decrease of approximately 28% in the TG concentrations when comparing the baseline with 12 months after treatment. In contrast, there were no significant differences in the concentrations of LDL, TC, and HDL before and after treatment. Infections and inflammation affect TG metabolism in humans and animals, causing hypertriglyceridemia.<sup>26</sup> Although the role of TG in atherosclerosis has not been a major focus in atherosclerosis research, important clinical studies have shown recently that prolonged hypertriglyceridemia increases the risk of myocardial infarction, ischemic heart disease, and death.<sup>27,28</sup> In addition, excess TG amplifies the risk of heart disease when combined with other risk factors, such as decreased plasma HDL levels. These studies demonstrated that patients with low HDL cholesterol and high TG levels have more extensive coronary atheromas than those with an isolated elevation of LDL cholesterol. There was a significant reduction of the TG/HDL ratio after 12 months of periodontal treatment.

Although the levels of LDL in plasma did not change with treatment, their nonlinear optical response changed after 12 months of periodontal treatment. The ZS experiment was performed with LDL isolated from each patient, using the same concentration of particles and under the same experimental conditions. A typical position-dependent normalized-transmittance ZS curve is presented in Fig. 1. As reported in a previous study,<sup>20</sup> the peak-to-valley amplitude, represented by the amplitude of the parameter  $\theta$ , evaluates the degree of modification of the LDL particles in the sample: the higher the value of  $\theta$ , the less modified the LDL particle is. The inset in Fig. 1 shows the typical time-dependence curves of the transmittances of the LDL, measured in two different positions of the sample along the  $z$ -axis, before and after the beam focus. The  $\theta$  values of all the patients before and 12 months after the periodontal treatment are represented in the scatter plot shown in Fig. 2.



**Table 1** Periodontal parameters before and after treatment, reported as medians and interquartile range.

Variable	Before	After treatment		
	Baseline	3 months	6 months	12 months
Age—years old	45.2	45.3 (9.8)	45.5 (9.7)	45.7 (9.7)
Gender (male: female)	18:22	—	—	—
Race or ethnic group -n (%)				
Asian	—	—	—	—
Black	4	—	—	—
White	36	—	—	—
Outros	—	—	—	—
BMI (kg/m <sup>2</sup> )	26.9 (3.0)	26.9 (3.0)	26.9 (3.0)	26.9 (3.0)
Number of teeth	23.5 (6.00)	21.0 (6.25)	21.0 (6.25)	21.0 (6.25)
Number of pockets (%)	39.3 (15.8)	1.8 (3.10)	1.8 (3.10)	1.8 (3.10)
Bleeding on probing (%)	76.0 (25.5)	18.0 (12.25)	18.5 (17.75)	19 (15.00)

BMI—body mass index; SD—standard deviation; Values in bold show differences between baseline, three-month, six-month, and 12-month followup periods.

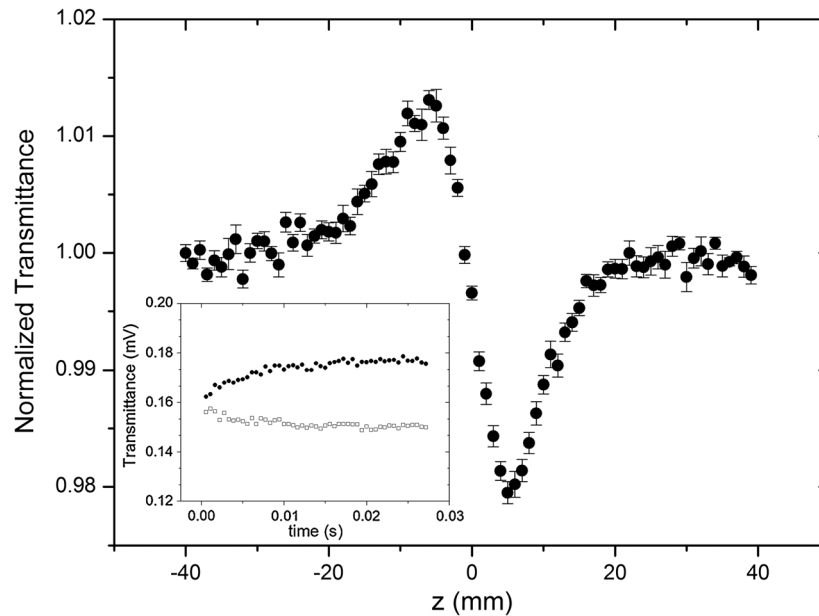
**Table 2** Plasma concentration of atherosclerosis risk markers, reported as medians and interquartile range or means and standard deviation depending on the distribution.

	Before	After treatment					
	Baseline	3 months	p-value baseline to 3 months	6 months	p-value baseline to 6 months	12 months	p-value baseline to 12 months
TC (mg/dL) <sup>a</sup>	204.0 (84.0)	192.50 (66.50)	n.s	189.00 (69.75)	n.s	196.50 (54.75)	n.s
TG (mg/dL) <sup>a</sup>	118.00 (68.0)	108.50 (55.50)	n.s	99.00 (38.25)	<b>0.03</b>	89.00 (31.00)	<b>0.01</b>
HDL (mg/dL)	47.4 (7.9)	48.30 (7.9)	n.s	48.10 (7.60)	n.s	48.70 (7.50)	n.s
LDL (mg/dL)	131.3 (40.8)	124.30 (36.10)	n.s	125.6 (34.70)	n.s	124.8 (32.20)	n.s
TG/HDL <sup>a</sup>	2.51 (1.19)	2.19 (1.36)	n.s	2.05 (1.03)	n.s	1.96 (0.89)	<b>0.04</b>
Anti-oxLDL (units)	1.01 (0.24)	0.99 (0.22)	n.s	0.95 (0.14)	<b>0.03</b>	0.89 (0.13)	<b>0.01</b>
CRP (mg/mL)	1.50 (0.55)	1.28 (0.36)	<b>0.003</b>	1.18 (0.20)	<b>0.001</b>	1.23 (0.22)	<b>0.002</b>

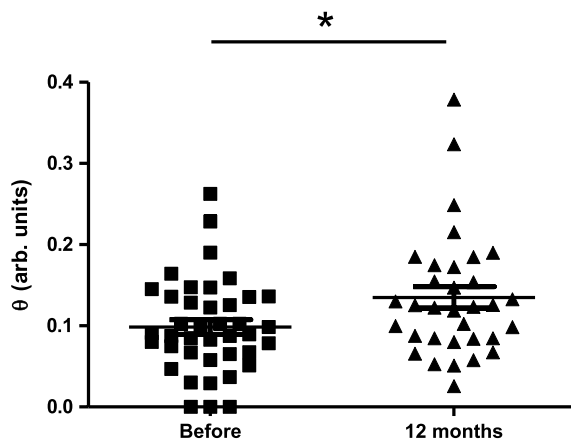
<sup>a</sup>Variable not normally distributed, expressed as median and interquartile range. TC—total cholesterol; TG—triacylglycerol; HDL—high-density lipoprotein; LDL—low-density lipoprotein; TG/HDL—ratio of triacylglycerol/high-density lipoprotein; hsCRP—high sensitivity C-reactive protein; n.s.—not significant. Values in **bold** show differences between baseline, three-month, six-month, and 12-month followup periods.

The mean-value of  $\theta$  from patients 12 months after treatment is about 37% higher than that before treatment. Our results indicate that patients with periodontitis present more modified LDL particles before treatment than they do 12 months after treatment ( $p$ -value = 0.04). In other words, associating the risk of developing cardiovascular disease with the concentration of oxidized LDL in the blood, our Z-scan results indicated that periodontal treatment improved the oxidative status of LDL. To support our

conclusions, we studied a particular case of a patient with severe alveolar bone loss around the teeth and frequent spontaneous bleeding. The position-dependent ZS curves of the LDL solutions from this patient before and three, six, and 12 months after treatment are shown in Fig. 3. The curves for three and six months after treatment are essentially flat, without peak-to-valley measurable amplitude. However, 12 months after treatment, the ZS typical curve of the LDL solutions from the patient



**Fig. 1** Typical position-dependent normalized-transmittance ZS curve of a patient. Inset—typical time-dependence curves of the transmittances of the LDL solutions extracted from patients, measured in two different positions of the sample, before (●) and after (□) the beam focus.

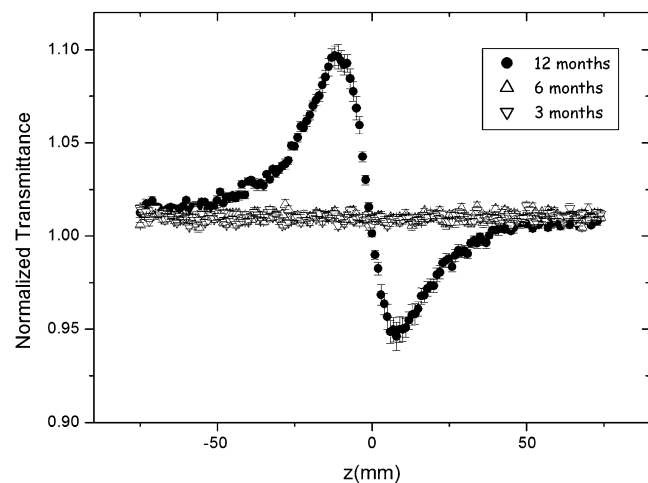


**Fig. 2** Scatter plot of the  $\theta$  values from all the patients before and 12 months after the periodontal treatment. \* $P < 0.05$ .

presented a significant change, with a well-defined peak-to-valley shape, as shown in Fig. 3.

The levels of oxLDL IgG antibodies were also investigated before and after periodontal treatment. As shown in Table 2, six months after periodontal treatment, the levels of oxLDL antibodies were decreased compared with those before treatment ( $p$ -value = 0.03). Case-control studies have demonstrated that elevated anti-oxLDL titers are indicative of myocardial infarction.<sup>29,30</sup> In our previous study, we showed that periodontal infection could increase the production or levels of antibodies against modified LDL. Because periodontal treatment reduced the amount of the modified LDL antigen, the levels of antibodies against oxLDL decreased as part of a modulation of the overall humoral immune response.

The effect of periodontal treatment on the levels of plasma cytokines and systemic inflammation markers was investigated. HsCRP, IL-6, and IL-8 levels were decreased three months after treatment ( $p$ -value = 0.003,  $p$ -value < 0.0001 and  $p$ -value =



**Fig. 3** Position-dependent normalized-transmittance ZS curves three (▽), six (Δ), and 12 (●) months after periodontal treatment. LDL from a patient with severe alveolar bone loss around the teeth and frequent spontaneous bleeding.

0.02, respectively). These levels are shown in Tables 2 and 3. The periodontal treatment had no effect on the levels of IL-1 $\beta$  ( $p$ -value = 0.48), IL-10 ( $p$ -value = 0.81), and TNF- $\alpha$  ( $p$ -value = 0.74), as shown in Table 3. IL-6 is cytokine that induces the hepatic acute phase response; it could affect TG clearance and/or production.<sup>31</sup> However, study in animals has shown that inflammatory cytokines decrease blood lipid levels, and that anti-cytokine treatment increases blood lipid levels until normalization.<sup>32</sup> Our results showed a decrease in IL-6 levels in the plasma with periodontal treatment, supporting the relationship between TG levels and IL-6 plasma concentrations.

CRP is another widely studied inflammatory marker in the relationship between cardiovascular and periodontal diseases. Epidemiological studies have shown that CRP levels are increased in healthy individuals with periodontal problems.<sup>33,34</sup>

**Table 3** Analysis of circulating peripheral white blood cells and plasma cytokine concentrations of individuals with chronic periodontitis disease before and after periodontal treatment.

	Before		After treatment						
	Baseline	3 months	<i>p</i> -value baseline to 3 months	6 months	<i>p</i> -value 3 to 6 months	<i>p</i> -value baseline to 6 months	12 months	<i>p</i> -value 6 to 12 months	<i>p</i> -value baseline to 12 months
Leukocytes ( $10^3$ cells/mm <sup>3</sup> ) <sup>a</sup>	7.1 (1.65)	6.1 (0.8)	<b>0.0001</b>	66.1(0.8)	n.s	<b>0.0001</b>	6.1 (0.75)	n.s	<b>0.0001</b>
Monocytes (cells/mm <sup>3</sup> )	477.5 (147.7)	486.0 (107.5)	n.s	461.5 (95.9)	n.s	n.s	500.5 (99.7)	n.s	n.s
Neutrophils ( $10^3$ /mm <sup>3</sup> )	4.2(1.2)	3.3(0.5)	<b>&lt;0.0001</b>	3.2(0.4)	n.s	<b>&lt;0.0001</b>	3.2(0.4)	n.s	<b>&lt;0.0001</b>
Lymphocytes ( $10^3$ /mm <sup>3</sup> )	2.0 (0.5)	2.0 (0.3)	n.s	2.0 (0.3)	n.s	n.s	2.1(0.3)	n.s	n.s
Eosinophils (cells/mm <sup>3</sup> ) <sup>a</sup>	146.5 (189.2)	136.0 (125.0)	n.s	177.5 (120.0)	n.s	n.s	162.5 (115.5)	n.s	n.s
Basophils (cells/mm <sup>3</sup> ) <sup>a</sup>	26.0 (55.0)	19.5 (57.0)	n.s	16.0 (64.5)	n.s	n.s	24.5 (62.2)	n.s	n.s
IL-1 $\beta$ (pg/mL) <sup>a</sup>	1.32 (0.05)	1.32 (0.07)	ns	1.31 (0.08)	ns	ns	1.31 (0.08)	ns	ns
IL-6 (pg/mL)	1.64 (0.11)	1.57 (0.05)	<b>&lt;0.0001</b>	1.52 (0.05)	ns	<b>&lt;0.0001</b>	1.51 (0.03)	ns	<b>&lt;0.0001</b>
IL-8 (pg/mL)	4.45 (0.32)	4.30 (0.24)	<b>0.02</b>	4.21 (0.22)	ns	<b>0.01</b>	4.21 (0.18)	ns	<b>0.01</b>
IL-10 (pg/mL)	3.59 (0.001)	3.59 (0.001)	ns	3.59 (0.001)	ns	ns	3.59 (0.001)	ns	ns
TNF- $\alpha$ (pg/mL)	1.70 (0.17)	1.68 (0.14)	ns	1.70 (0.16)	ns	ns	1.71 (0.16)	ns	ns

<sup>a</sup>Variable not normally distributed, expressed as median and interquartile range. Total and differential white blood cell count were measured in the blood; the levels of cytokines were in plasma. n.s—not significant. Values in **bold** show differences between baseline, three-month, six-month, and 12-month followup periods.

A moderate increase in CRP levels in individuals with periodontitis has led to the hypothesis of its association with cardiovascular disease. Thus, our study confirms the data found in the literature, because periodontal treatment significantly reduced the serum concentrations of hs-CRP.

As shown in Table 3, leukocyte and neutrophil counts were decreased three months after periodontal treatment (*p*-value < 0.0001 and *p*-value < 0.0001, respectively). No difference was found in the counts for monocytes (*p*-value = 0.34), lymphocytes (*p*-value = 0.64), eosinophils (*p*-value = 0.43), or basophils (*p*-value = 0.92) after periodontal treatment. These counts are also shown in Table 3. Studies have revealed that a high concentration of leukocytes is a risk factor for cardiovascular disease, due to an association with an inflammatory response. This idea is supported by the presence of leukocytes in atherosclerotic plaques<sup>35</sup> and by the fact that leukocyte count is a predictor of vascular disease.<sup>36</sup> In our previous studies, we have shown that patients with periodontitis had more leukocytes than the control group.<sup>25</sup> The increased leukocyte count was attributed to higher neutrophil counts. Recently, more attention has been drawn to the neutrophil count in investigations on the pathogenesis of atherosclerosis. The increase in neutrophil count may reflect the burden of atherosclerosis and tissue damage.<sup>37</sup> In addition, these cells can be found in aortic lesions and plaques in cerebral arteries.<sup>38</sup> In this present study, the elimination of periodontal infection after periodontal treatment resulted in a progressive decrease in the number of circulating leukocytes and in the number of neutrophils. Corroborating with the decrease in the number of neutrophils, there was a significant decrease in the plasma concentration of IL-8, a known atherosclerosis-associated cytokine, after periodontal treatment.<sup>39</sup>

## 4 Conclusions

The successful treatment of patients with chronic periodontal disease induces systemic changes in several markers, reflecting a decrease in their cardiovascular risk for atherosclerosis. Here, these changes have been divided into early and late events. The early events include a rapid reduction in inflammatory cytokine levels and leukocyte and neutrophil counts. The late events include changes in TG and anti-oxLDL antibody levels and the improvement of the quality of LDL particles evaluated by using the Z-scan technique. We therefore conclude that when cardiovascular intervention or prevention is performed, great care has to be taken in the selection of markers used to evaluate patients. In particular, the ZS nonlinear optical response in the milliseconds time-scale regime was shown to be a novel and complementary tool to evaluate the success of periodontal treatment. Furthermore, these results illuminate the paramount importance of oral health in cardiovascular prevention.

## Acknowledgments

The authors thank Silvana A. da Silva from the institute of biomedical sciences, university of são paulo, for technical support. This study was supported by grants from FAPESP, INCT-FCX, and INCT-INAMI. The authors report no conflict of interest related to this study.

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