

# Effect of Non-surgical Periodontal Therapy on Levels of Inflammatory Markers in Gingival Crevicular Fluid and Serum of Patients With Breast Cancer and Periodontitis.

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## Research Article

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# Abstract

**Purpose:** studies have demonstrated the positive impact of non-surgical periodontal therapy (NSPT) on the control of local and systemic infection/inflammation in normosystemic and systemically compromised patients, represented by the improvement of periodontal clinical parameters and reduction in the levels of inflammatory markers in the gingival crevicular fluid (GCF), saliva and serum. This study aimed to evaluate periodontal clinical parameters and inflammatory mediators in GCF and serum, before and after NSPT, in patients with periodontitis and breast cancer, before chemotherapy.

**Methods:** seventeen women with histopathological diagnosis of invasive ductal carcinoma and periodontitis were submitted to the evaluation of clinical periodontal parameters (plaque index – PI, bleeding on probing – BOP, probing depth – PD, clinical attachment level – CAL) and submitted to scaling and root planing (SRP), at an interval of 24 hours. At the beginning of the study (*baseline*), before NSPT, samples of tumor microenvironment fluid (TM), GCF and peripheral blood (serum) were collected for the determination of inflammatory markers IL-1 $\beta$ , TNF- $\alpha$ , TGF- $\beta$  and IL-17, using the LUMINEX methodology. Seven days after SRP, new GCF and serum samples were obtained and analyzed.

**Results:** TGF- $\beta$  levels were significantly decreased in GCF and serum ( $p < 0.05$ ), while IL-17 concentrations were statistically reduced in GCF ( $p < 0.05$ ).

**Conclusion:** NSPT decreased local and systemic inflammatory markers and may be an important tool in the multidisciplinary approach of women with breast cancer and periodontitis before chemotherapy.

## Structured Abstract (Statement Of Relevance)

Periodontitis are not only restricted to the oral cavity and can affect the systematic health of an individual. Epidemiologic studies have suggested a positive association between periodontal disease and cancer risk in the different tissues, including breast cancer. To our knowledge, this is the first time that the inflammatory markers TGF- $\beta$ , IL-17, IL-1 $\beta$  and TNF- $\alpha$  were systemically evaluated, through peripheral blood (serum), before and after non-surgical periodontal treatment (NSPT) in women with breast cancer and periodontitis, before chemotherapy. Our results provide unpublished information and raise important perspectives suggesting that NSPT is preferred before chemotherapy. Systemic decrease in the inflammatory mediators' levels could be understood as an important adjuvant for antitumor therapy since it has been discussed that interference in cytokine-mediated signaling pathways can bring benefits and prevent possible metastasis.

## Introduction

Periodontitis is a multifactorial chronic disease associated with a dysbiotic biofilm and characterized by the progressive destruction of the tooth-supporting apparatus. This high prevalence disease has been related to tooth loss, with negative impacts on masticatory function, aesthetics, general health and quality of life, thus considered as a significant public health problem [1,2]. There is relevant evidence of

the association between periodontitis and other systemic conditions including cardiovascular diseases, lung diseases and gestational complications. This can be explained by the fact that periodontal bacteria and inflammatory mediators are not restricted at the gingival level. Once in the bloodstream, they spread throughout the body, causing a measurable impact on systemic inflammation [3,4].

In the immunopathogenesis of periodontitis, a significant production of inflammatory markers is observed, including cytokines IL-1 $\beta$ , TNF- $\alpha$ , TGF- $\beta$  and IL-17 [5,6]. In this context, IL-1 $\beta$ , TNF- $\alpha$  and IL-17 have been associated with periodontal destruction, increased bone resorption and connective tissue degradation by stimulating the production of prostaglandins and collagenases, besides being able to modulate systemic diseases [6,7]. Growth transformation factor-beta (TGF- $\beta$ ) is a multifunctional cytokine that plays a modulating role, dysregulates the transcription of other pro-inflammatory cytokines, increases tissue repair, accelerates connective tissue remodeling and promotes angiogenesis. The absence of TGF- $\beta$  contributes to major periodontal destruction. Therefore, higher levels of TGF- $\beta$  in GCF occur to minimize the extent of the injured tissue as the disease progresses [8].

Breast cancer is the second most frequent type of cancer in the world and the first among women, accounting for 25% of new cases each year. However, the association of this condition with periodontitis is still unknown [9]. It has been suggested that inflammation associated with cancer is similar to chronic inflammations. The key factors of inflammation may play a role in tumor suppression by stimulating the antitumor immune response. However, they often seem to stimulate the development of cancer [10]. As regards breast cancer, the soluble mediators present in the tumor microenvironment (IL-1 $\beta$ , TNF- $\alpha$ , TGF- $\beta$  and IL-17) are related to the worst prognosis of cancer due to pro-tumorigenic activities such as angiogenesis, proliferation, local tumor invasion, tumor growth and metastasis, especially in the bone and lung [11-13]. Currently, the scientific community has proposed adjuvant therapeutic strategies that aim to block intracellular signaling pathways mediated by these cytokines and thus prevent metastasis and improve the cancer prognosis [12,13].

There is strong scientific evidence of the positive impact that non-surgical periodontal therapy (NSPT), especially scaling and root planing (SRP), in the control of local and systemic infection/inflammation in normossystemic or systemically compromised patients represented by the improvement of clinical periodontal parameters, decreased count of periodontopathogenic bacteria and reduced levels of inflammatory markers in gingival crevicular fluid (GCF), saliva and serum [3, 4]. Recently, Vargas-Villafuerte et al.[14] have shown that NSPT can reduce inflammatory markers in women with breast cancer and periodontitis, hence preventing exacerbation of the periodontal disease and sepsis in the face of immunosuppression caused by chemotherapy [15]. The reduction in serum markers achieved by the treatment of periodontitis can be understood as especially important when preceded by chemotherapy, since it could contribute to a more favorable response to antitumor therapy, thus improving the prognosis of these women. Thus, this study aimed to evaluate the impact of NSPT, focused on SRP, on the levels of IL-1 $\beta$ , TNF- $\alpha$ , TGF- $\beta$  and IL-17 in GCF and serum of women with periodontitis and breast cancer, before chemotherapy.

# Materials And Methods

## Study Population

A total of 17 women with invasive ductal carcinoma and periodontitis, according to the international classification of periodontal diseases adopted by the American Academy of Periodontology (AAP) were recruited from the Department of Gynecology and Obstetrics of Medicine Clinics Hospital, Ribeirão Preto School of Medicine - University of São Paulo (HCFMRP-USP), between March/2017 and January/2018. The study protocol was approved by the Institutional Ethics Committee (protocol 37030514.7.0000.5419). All participants signed a written informed consent (IC) form in accordance with the Helsinki Declaration of 1964 (revised in 2013).

## Inclusion and Exclusion Criteria

Inclusion criteria were as follows: 1) age  $\geq 35$  years; 2) minimum 10 teeth, excluding third molars and teeth with extraction indication; 3) clinical attachment level (CAL)  $\geq 5$  mm and probing depth  $\geq 5$  mm in proximal sites of at least two non-adjacent teeth [16]. Exclusion criteria were as follows: 1) smoking; 2) diabetes mellitus; 3) pregnancy; 4) history of antibiotic therapy and/or periodontal treatment in the last six months; 5) use of bisphosphonates or any medication that could interfere with periodontal aspects; 6) extensive prosthetic involvement; 7) previous history of cancer and chemotherapy; 8) cardiovascular diseases; 9) history of immunosuppression.

## Experimental Design

The patients were invited to participate in the study at the time of biopsy to confirm breast cancer. Samples of the tumor microenvironment (TM) fluid were collected while a biopsy was performed. Clinical and histological parameters related to cancer were obtained after the final biopsy report.

Clinical periodontal parameters (PI, BOP, PD, CAL) and biological fluids (TM, GCF and serum) were evaluated at baseline, which corresponded to the beginning of the study, before non-surgical periodontal treatment (NSPT) and chemotherapy and /or surgical for the tumor. NSPT was performed within a 24-hour interval by a specialist in periodontics (FTD). Seven days after the NSPT, new samples of GCF and serum were collected. The samples were stored at a temperature of  $-80^{\circ}\text{C}$  until the moment of dosing.

## Examiner Calibration

Clinical parameters were measured by a single calibrated Examiner (FTD). For calibration, duplicate measurements of PD and CAL, at 48 hours intervals, were obtained from 10 patients unrelated to the study who presented at least two pairs of contralateral teeth with CAL  $\geq 5$  mm at proximal sites. Calibration was accepted when the percentage of agreement was  $> 90\%$  between the measurements.

## Clinical Assessment

Clinical periodontal parameters were evaluated using the North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA) at baseline. Teeth counting was performed. The plaque index (PI) was evaluated in a

dichotomous way by considering the presence or absence of visible biofilm in the gingival margin on tooth surfaces (buccal, lingual/palatine, mesial and distal) [17]. BOP represented the presence or absence of bleeding in six sites per tooth, three per vestibular (mesio-buccal, buccal and disto-buccal) and three per lingual/Palatine (mesio-lingual/palatine, lingual/palatine and disto-lingual/palatine), up to 15 seconds after the completion of the probing depth. PI and BOP were expressed in percentage [18]. PD (measured between the gingival margin and the bottom of the gingival groove or periodontal pocket) and CAL (measured the distance between the cement-enamel junction to the most apical portion of the periodontal pocket/groove) were calculated in millimeters (mm) at six sites per tooth.

Data related to breast cancer were recorded in the electronic and manual records of each patient, including tumor type, histological grade (grade 1, 2 and 3), expression of estrogen receptors (ER), progesterone (PR) and expression of HER2 protein.

## Collection TM Samples

During the *core biopsy*, four fragments of the breast tumor tissue were removed with a thick-gauge needle attached to a special pistol. This procedure was ultrasound-guided and performed under local anesthesia. After the removal of each tissue fragment, and before its storage in a container, the biopsied material was placed on a sterile gauze and the fluid samples were obtained using Periopaper<sup>®</sup> strips (Oralflow Inc., Amityville, NY, USA). One collection was performed for each fragment, with a total of four samples for each patient. The strips were carefully inserted next to the tissue fragment for 15 seconds and then placed in sterilized *Eppendorf* tubes. These samples were stored at a temperature of -80°C, until laboratory processing.

## Collection GCF Samples

At baseline and after seven days of SRP, GCF samples were collected. The supragingival plaque at the collection sites was removed and carefully dried with air jets, then isolated with sterile cotton rolls for no saliva interference. Samples were obtained from four specific interproximal sites (two anterior and two posterior teeth), and 3 samples were collected from each site, using Periopaper<sup>®</sup> strips (Oralflow Inc., Amityville, NY, USA), totaling 12 strips for each patient. The collection sites had a probing depth  $\geq 5$  mm and positive probing bleeding, and the same sites were used as a reference at all collection time points. The strips were carefully inserted near the edge of the gingival groove for 30 seconds, then placed in sterilized *Eppendorf* tubes and stored at a temperature of -80°C, until the moment of laboratory quantification. Absorbent paper strips contaminated with blood were discarded.

## Blood Sampling (serum)

From each patient, in the collection time points (*baseline* and after seven days), 10mL of peripheral blood was collected by venous puncture and stored in tubes (BD Biosciences) containing an inert gel that separates the serum and the blood clot and no surface treatment. The collection procedure respected the norms of institutional biosafety guidelines and was carried out by properly trained professionals. After the puncture, the samples were kept for one hour at room temperature for clot retraction. After this period,

the samples were taken to a centrifuge and subjected to 3000 rpm (rotation per minute), for 8 minutes. Using pipettes, 1.5mL of serum aliquots were transferred to properly identified sterilized *Eppendorf* tubes and stored at a temperature of -80°C until the time of analysis.

## Non-surgical periodontal treatment (NSPT)

At first, all women received instructions for effective plaque self-control, including information on Bass brushing technique and interproximal cleaning with dental floss and interdental brushes [19]. They were also instructed to use soft bristle brushes and motivated to brush the tongue dorsum at least once a day. The participants were subjected to full-mouth SRP at 24 hours intervals, using manual instruments (Gracey curettes, Hu-Friedy, Chicago, IL, USA) and ultrasound. Finally, coronary polishing with rubber cups was performed on all dental elements followed by fluoride topical application. At the end of the study, all women were referred to a Supportive Periodontal Therapy (SPT) program.

## Biochemical Analysis

The quantification of cytokines (IL-17, TNF- $\alpha$ , IL-1 $\beta$  and TGF- $\beta$ ) in the three fluids was performed using commercially available kits (HTH17MAG-14k and TGFBMAG-64k – 01-Milliplex™ map, Merck Millipore Headquarters, Billerica, MA, USA) in a MAGPIX® analyzer (Luminex Corporation, Austin, TX, USA). 96-well plate assay was performed according to the manufacturer's instructions. Briefly, the filter plate was moistened with the *washing buffer*, and then the solution was aspirated from the wells. Magnetic *beads* coated with monoclonal antibodies to the analytes were added to the wells. The samples and patterns were transferred to the wells and incubated *overnight* (16-18 hours), at 4°C. The wells were washed again and a mixture of biotinylated secondary antibodies was added. After one hour of incubation, Streptavidin R-Phycoerythrin was added to the wells, and this set was incubated for another hour. A washing step was performed to remove unbound reagents. Then, sheath fluid (Luminexs, MiraiBio, Alameda, CA, USA) was added to the wells. The plates were then analyzed by MAGPIX®, for obtaining the average fluorescence intensity. Samples below the detection limit were recorded as zero. All samples were individually analyzed and cytokine levels were estimated from a fifth-degree polynomial curve using xPONENT® *software* (Luminex Corporation, Austin, TX, USA). The values found for each of the cytokines were standardized by sample volume. Thus, the results were expressed in pg/mL.

## Statistical Analyses

The sample size was calculated considering a 50% difference in the GCF mean levels of the biochemical markers. It was assumed that the standard deviations are at most 80% of the mean values and a power of 90% ( $p = 0.05$ ) was accepted. Statistical analyses performed with SPSS 22.0 software (Statistical Package for Social Sciences) and graphs were obtained in GraphPad Prism 5.0 software for Windows. The patient was considered as the statistical unit ( $n = 17$ ) and a significance level of 5% ( $p < 0.05$ ) was adopted. In the descriptive analysis, data of the variables analyzed were reported as mean, standard deviation, median, interquartile range, absolute frequency and their respective percentages. The Wilcoxon test was performed and graphs were presented in *box-plot*.

## Results

The descriptive analysis of the evaluated variables is available in Table 1. The mean age of the patients was 52 years and 22 dental units in average. Clinical periodontal parameters were compatible with generalized periodontitis stage III grade B (Table 1). All women (n=17) were climacteric and had a primary diagnosis of invasive ductal carcinoma. Most patients (n=14) displayed grade 2 tumor stage at the time of diagnosis, positive estrogen and progesterone receptors (RE n=14 / RP n=10) positive and negative HER2 gene (N=10) (Table 2).

At the beginning of the study, IL-1 $\beta$ , TNF- $\alpha$ , TGF- $\beta$  and IL-17 was observed in the three environments analyzed (Table 3).

After one week of SRP, it was possible to observe that TGF- $\beta$  and IL-17 levels significantly reduced in GCF ( $p < 0.05$ ). Also, TGF- $\beta$  concentrations were reduced in serum ( $p < 0.05$ ). As regards IL-1 $\beta$  and TNF- $\alpha$  levels in the GCF and serum concentrations of IL-1 $\beta$ , TNF- $\alpha$  and IL-17, no significant reductions were observed ( $p > 0.05$ ) (Table 4).

Figure 1 and Figure 2 show box-plot graphs with values for each of the variables evaluated, at baseline and one week after NSPT, in GCF and serum, respectively.

## Discussion

This study provides important and unpublished information on the impact of NSPD on reducing levels of local and systemic inflammatory markers in women with periodontitis and breast cancer when performed before chemotherapy. This finding raises two important perspectives suggesting periodontal treatment is preferred before chemotherapy. First, the elimination of infection/inflammation foci would substantially reduce the risk of acute lesions for the patient, such as mucositis, and, consequently, bacteremia (sepsis) during the antineoplastic treatment process [14,15]. Second, and directly related to our results, would be the fact that a systemic decrease in the inflammatory mediators' levels could be understood as an important adjuvant for antitumor therapy since it has been discussed that interference in cytokine-mediated signaling pathways can bring benefits and prevent possible metastasis [12,13,20,21]. In GCF samples, TGF- $\beta$  and IL-17 concentrations were statistically reduced ( $p < 0.05$ ) one week after SRP. Decreased TGF- $\beta$  levels after NSPT ( $p < 0.05$ ) in GCF was found by Pamuk et al. [22]. These results were similar to our study. Escobar et al. [23] observed a reduction in TGF- $\beta$  levels in GCF after seven days of SRP. Regarding IL-17, Mistry et al. [24] also found significant decreases of this cytokine in GCF after three months of SRP, associated or not with antimicrobial photodynamic therapy. Thus, validating the role of NSPT in the control of local inflammation, regardless of the treatment modality.

Also, TGF- $\beta$  levels were reduced in serum before and after NSPT ( $p < 0.05$ ). Khalaf et al. [25] showed that TGF- $\beta$  levels are higher in serum, GCF and saliva of patients affected by periodontitis when compared to healthy controls, and this marker may influence systemic inflammatory disorders. High concentrations of this cytokine have been associated with a worse prognosis of breast cancer because it is involved in

processes that stimulate tumor promotion and metastases, such as angiogenesis and immune system evasion [26,27]. Recently, researchers have defended the idea that blockages of TGF- $\beta$  signaling pathways, through biological or pharmacological inhibitors, may be promising immunotherapy for breast cancer treatment [28,29].

Although no statistically significant ( $p>0.05$ ), decreased levels of cytokines IL-1 $\beta$  and TNF- $\alpha$  in GCF were observed. Previous data found a significant decrease in these markers in GCF after NSPT, in normosystemic patients [30,31]. Although the difference was not significant, a trend towards a reduction in these markers was observed. Similarly, serum concentrations of IL-1 $\beta$ , TNF- $\alpha$  and IL-17 showed a slight tendency to decrease ( $p>0.05$ ). Previous studies that assessed clinical periodontal parameters and biomarkers in serum, before and after NSPT evidenced improvement in clinical parameters and diminished IL-1 $\beta$ , TNF- $\alpha$  and IL-17 levels in normosystemic patients with periodontitis [32,33].

Particular attention should be given to these three inflammatory markers (IL-1 $\beta$ , TNF- $\alpha$  and IL-17), since they have been associated with processes that lead to tumor expansion and metastasis and, consequently, to a worse prognosis of malignant neoplasm of the breast.[11,34,35]. Therefore, recently, the scientific community has proposed adjuvant therapeutic strategies that aim to block intracellular signaling pathways mediated by these cytokines and thus prevent metastasis and improve the prognosis of cancer [12,13,20,21].

Likewise, the present study quantified the four inflammatory markers in three different environments, and identified them at baseline and one week after NSPT in GCF and serum. Due to ethical issues, TM samples could only be obtained at the time of biopsy. These cytokines have been commonly related to immunopathogenesis of periodontitis and breast cancer and our data corroborate the idea that inflammation associated with cancer is similar to that observed in chronic inflammation, especially concerning Th1, Th2 and Th17 responses [5,10,12]. For Freudenheim et al. [9], the presence of periodontopathogenic bacteria in the region of the breast tumor and mediators of chronic inflammation plays an active role in carcinogenesis and could explain an association between both diseases. Güven et al. [36] and Jia et al. [37] highlighted that immunoinflammatory mechanisms may be responsible for increasing the chances of cancer development in those individuals who have periodontal disease.

Both diseases are prevalent diseases in adults over the age of 35 [38]. In the present study, the mean age for women was 52.4 years. We chose to maintain the same age pattern in the selection of patients to avoid interference of this factor in the results. Another relevant aspect was the lower number of teeth in the population studied, accounting for an average of 22 dental units. Many studies have assessed the association of periodontal diseases and cancer and performed a correlation between tooth loss with increased risk of malignant neoplasms [39, 40]. Amódio et al. [41] and Vargas-Villafuerte et al. [42] also found fewer dental units remaining in a population with the same characteristics as the subjects evaluated in our study.

In this study, periodontal parameters characterized a similar pattern of periodontitis, represented by higher values of PI, PD, CAL, and BOP, when compared to periodontically healthy individuals [43].

Therefore, patients were diagnosed with generalized periodontitis stage III grade B [1]. It is worth mentioning that, although periodontitis is associated with many systemic conditions [4], women with other diseases, besides invasive ductal carcinoma, were not included to avoid interference in the results.

The data related to the diagnosis of breast cancer showed that the patients had a similar pattern of neoplasia, as 100% presented a diagnosis of invasive ductal carcinoma and absence of metastasis at the time of collection. Most women had a more favorable course of the disease (Table 2). The histological grades are based on differentiation from normal breast cells. Grade 1 represents cells with normal appearance and slow growth, grade 2 with mixed characteristics and grade 3 with abnormal cells of rapid and aggressive growth. These cells may or may not have receptors for estrogen and progesterone, hormones that stimulate their growth. The presence of receptors is related to slow tumor growth and better response to hormone therapy compared with the absence of receptors. In addition, breast cancers may have a protein excess that promotes their growth, which corresponds to HER2. Women with HER2-positive breast cancer have faster and more aggressive tumor growth [44].

In this research, women diagnosed with cancer for the first time and not undergoing previous treatment (chemotherapy, radiotherapy or mastectomy) were chosen to avoid the interference of these therapies in periodontal and systemic conditions, reducing the number of biases and impaired results. Antineoplastic treatment side effects may alter oral cavity and systemic conditions of individuals. For example, the direct stomatotoxicity of chemotherapy drugs can affect the gingiva by inducing marginal gingivitis and periodontitis or aggravating pre-existing periodontal conditions, besides the risk of systemic complications resulting from immunosuppression [42,45].

On the other hand, the small sample size may have contributed to the results found and constituted a limitation of this study. Many women did not agree to participate due to the psychological issues associated with breast cancer. It is suggested that future long-term studies follow the effects of NSPT in women with breast cancer and correlate the findings with the prognosis and response to antineoplastic treatment.

Thus, NSPT can be an important tool in the multidisciplinary approach of women with breast cancer and periodontitis before chemotherapy, as it decreased TGF- $\beta$  levels in serum and GCF and IL-17 concentrations in GCF of these patients.

## **Declarations**

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The authors declare no conflict of interest.

The authors have no relevant financial or non-financial interests to disclose.

**Availability of data and material:**

Patients have given prior written consent and the aspect of confidentiality were respected.

**Code availability:**

Not applicable

**Authors' Contributions:**

Felipe Torres Dantas – Study design, collection samples, non-surgical periodontal therapy, biochemical analysis, data analysis, manuscript writing and manuscript agreement.

Pedro Henrique Felix Silva - Biochemical analysis, clinical assessment, manuscript writing and manuscript agreement.

Hélio Humberto Angotti Carrara - Recruitment of patients, core biopsy, collection samples and manuscript agreement.

Francisco Jose Candido dos Rei – Study conception, recruitment of patients, core biopsy and manuscript agreement.

Fabiani Gai Frantz - Biochemical and data analysis and manuscript agreement.

Michel Reis Messoro - Statistical and data analysis, manuscript critical review and manuscript agreement.

Flávia Aparecida Chaves Furlaneto Messoro - Data analysis, writing assistance, manuscript critical review and manuscript agreement.

Daniela Bazan Palioto - Study conception, study design, guidance to other authors, data analysis, writing assistance and manuscript agreement.

All authors read and approved the final manuscript.

**Ethics approval:**

The study was conducted according to the guidelines of the 1964 Declaration of Helsinki (revised in 2013) and approved by the Institutional Ethics Committee (Protocol 37030514.7.0000.5419 / approved in 12.14.2014).

### **Consent to participate:**

Informed consent was obtained from all individual participants included in the study

### **Consent to publish:**

The authors affirm that human research participants provided informed consent for publication of data.

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## Tables

Table 1  
Descriptive analysis of the evaluated variables (n = 17).

Variables	$\mu \pm \sigma^*$	Md (IQ)**
Age	52,47±9,88	50,00 (44,50 – 59,00)
Number of teeth	22,06±7,47	23,00 (19,00 – 28,00)
PI baseline	67,00±24,09	75,00 (43,00 – 86,00)
BOP Baseline	67,00±21,08	65,00 (51,00 – 84,00)
PD mm baseline	3,64±0,33	3,61 (3,44 – 3,99)
CAL mm baseline	3,92±0,34	3,90 (3,64 – 4,05)
* Mean and standard deviation; * * median (interquartile range); PI: plaque index; BOP: bleeding on probing; PD: probing depth; CAL: clinical attachment level.		

Table 2  
Grade distribution, ER, PR, HER2 of the participants evaluated (n = 17).

Variables	n (%)
<b>Grade</b>	
1	2 (11,80)
2	14 (82,40)
3	1 (5,90)
<b>RE</b>	
No	4(23,50)
Yes	13 (76,50)
<b>RP</b>	
No	7 (41,20)
Yes	10 (58,80)
<b>HER2</b>	
No	10(58,80)
Yes	7 (41,20)
ER: estrogen receptor; RP: progesterone receptor; HER2: human epidermal growth factor receptor 2.	

Table 3

Descriptive analysis of cytokine levels evaluated in the different environments at baseline.

<b>Variables</b>	<b><math>\mu \pm \sigma^*</math></b>	<b>Md (IQ)**</b>
<b>TM</b>		
<b>TGF-<math>\beta</math></b>	2801,47 $\pm$ 6359,69	703,50 (516,00 – 1569,75)
<b>IL-17</b>	15,95 $\pm$ 2,87	15,25 (13,85 – 18,17)
<b>IL-<math>\beta</math></b>	8,06 $\pm$ 2,61	7,15 (6,40 – 8,70)
<b>TNF-<math>\alpha</math></b>	23,16 $\pm$ 54,83	5,15 (4,27 – 14,87)
<b>GCF</b>		
<b>TGF-<math>\beta</math></b>	323,65 $\pm$ 84,44	344,00 (241,00 – 344,00)
<b>IL-17</b>	13,51 $\pm$ 2,57	12,60 (11,38 – 15,03)
<b>IL-<math>\beta</math></b>	186,37 $\pm$ 200,24	106,53 (36,93 – 327,00)
<b>TNF-<math>\alpha</math></b>	14,29 $\pm$ 7,09	14,57 (7,92 – 19,67)
<b>SERUM</b>		
<b>TGF-<math>\beta</math></b>	68299,41 $\pm$ 10839,53	69299,41 (60720,00 – 80160,00)
<b>IL-17</b>	3,42 $\pm$ 2,48	2,50 (2,20 – 3,66)
<b>IL-<math>\beta</math></b>	1,73 $\pm$ 1,24	1,43 (1,00 – 2,01)
<b>TNF- <math>\alpha</math></b>	6,14 $\pm$ 2,93	6,05 (3,49 – 7,21)
* Mean and standard deviation; * * median (interquartile range); TM: tumor microenvironment; GCF: gingival crevicular fluid; values in pg/mL.		

Table 4

Cytokine levels at baseline, after one week of NSPT and the difference ( $\Delta$ ) between the two time points in GCF and serum.

Variables	Baseline*	One week*	$\Delta^*$	p-value
<b>GCF</b>				
<b>TGF-<math>\beta</math></b>	323,65 $\pm$ 84,44	243,88 $\pm$ 11,88	79,76 $\pm$ 84,54	0,004 <sup>†</sup>
<b>IL-17</b>	13,51 $\pm$ 2,57	11,60 $\pm$ 1,00	1,91 $\pm$ 2,85	0,018 <sup>†</sup>
<b>IL-<math>\beta</math></b>	186,37 $\pm$ 200,24	97,98 $\pm$ 62,31	88,39 $\pm$ 211,40	0.255
<b>TNF-<math>\alpha</math></b>	14,29 $\pm$ 7,09	13,06 $\pm$ 10,64	1,22 $\pm$ 7,30	0.381
<b>SERUM</b>				
<b>TGF-<math>\beta</math></b>	68299,41 $\pm$ 10839,53	19151,31 $\pm$ 9383,78	49148,10 $\pm$ 10894,42	0,000 <sup>†</sup>
<b>IL-17</b>	3,42 $\pm$ 2,48	3,21 $\pm$ 2,83	0,21 $\pm$ 0,77	0.109
<b>IL-<math>\beta</math></b>	1,73 $\pm$ 1,24	1,71 $\pm$ 1,28	0,01 $\pm$ 0,14	0.572
<b>TNF-<math>\alpha</math></b>	6,14 $\pm$ 2,93	6,07 $\pm$ 2,87	0,07 $\pm$ 1,04	0.850
* Mean and standard deviation; <sup>†</sup> statistically significant (Wilcoxon Test, P<0.005); NSPT: non-surgical periodontal therapy; GCF: gingival crevicular fluid; values in pg/mL.				

## Figures

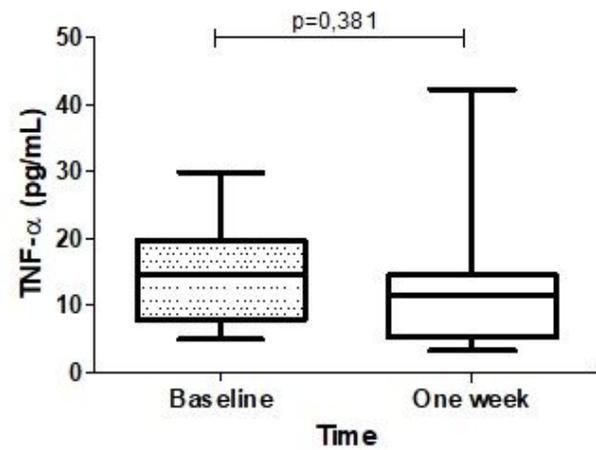
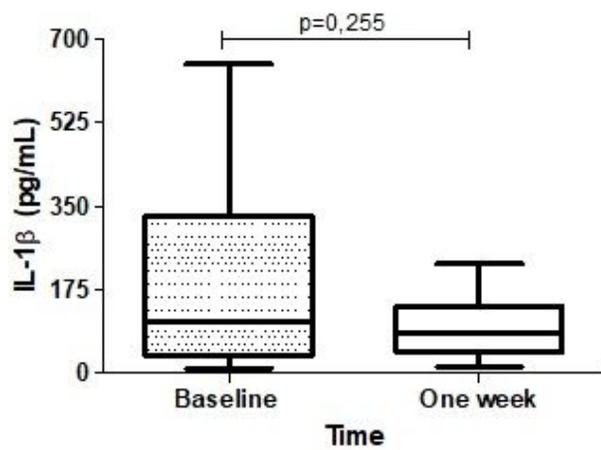
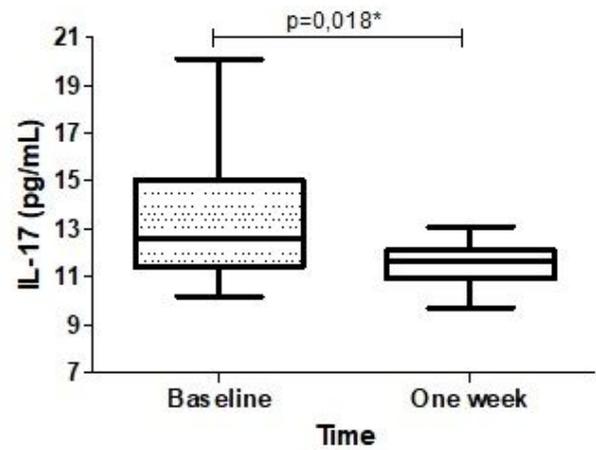
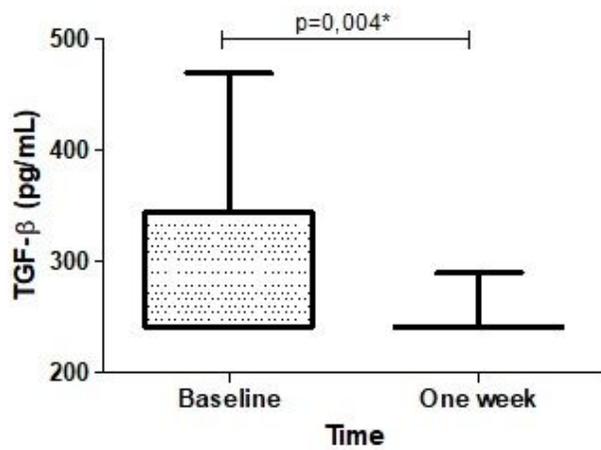
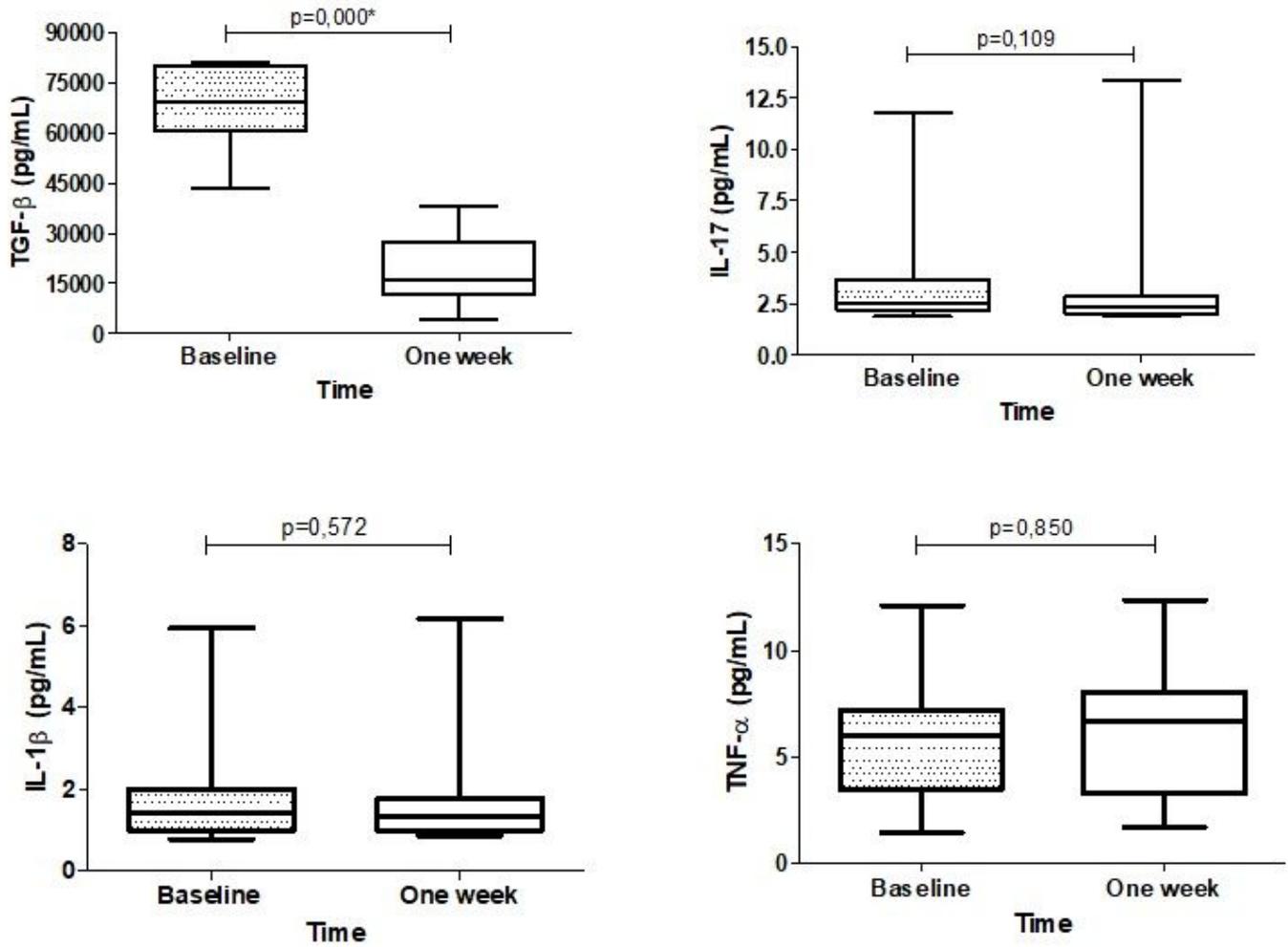


Figure 1

Box-plot of the cytokines values observed in the GCF at baseline and after one week. (\*statistically significant difference [ $p < 0.05$ ]).



**Figure 2**

Box-plot of serum cytokine values analyzed at baseline and after one week. (\*statistically significant difference [ $p < 0.05$ ]).