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Effects of Chemotherapy on the Leucocyte Infiltration in Periodontal Tissues of Cancer Patients: A Preliminary Study

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Abstract

Objective: We will investigate over the effects that chemotherapy can exert on T and B lymphocytes in periodontal tissue of tumor bearing patients undergoing treated using chemotherapy.

Methods: Twelve tumor bearing patients undergoing chemotherapy were selected before starting administration of the drug. Tissue samples of periodontium were collected before and after the end of chemotherapy and evaluated by immunohistochemistry, using antibodies against B and T cell antigens (CD20, CD3, CD4, CD8) and other populations (CD1a, S100, CD117, CD56). Statistical analysis was performed.

Results: After the administration of Rituximab, R-CHOP and Carboplatin-Docetaxel chemotherapy CD20+ B cells, CD3+ T cells and CD4+/CD8+ subpopulation were decreased. Same populations increased after administration of Cisplatin and Docetaxel. In one case, treated using Folfox, CD4+ T cells and CD8+ T cells are strongly decreased while absolute CD3+ T cell number remain stable.

Conclusions: Data obtained show important effects of chemotherapy on T and B cells of periodontal tissue suggesting involvement in periodontitis development that might be confirmed with other studies; if confirmed; it would be possible to formulate predictable strategies for the periodontal health of these patients.

Keywords: Chemotherapy; Periodontal diseases; Leucocytes; Side effects; Biopsy; Rituximab; Carboplatin; Docetaxel; Folfox

Introduction

Cancer is known to be a group of uncontrolled cells that grow and can invade and spread out in every site of the human body [1]. About 100 different types of cancer are described in today's literature and they can manifest with a lot of symptoms and signs.

Depending on type, location, TNM stadiation and patient preferences, clinicians have many treatment options, including surgery, radiation therapy and chemotherapy. The goal of therapy is the death of malignant cells that can be attained overall when aberrations in the mechanism that controls adaptive stress response making the target of drugs vulnerable [2,3].

Many different side effects are associated to chemotherapy and affect overall digestive tract, reproductive system, nervous system and bone marrow.

The reduced bone marrow function is due to the depletion of stem cell pool from which consequently originates a lower number of cells.

The immune system is one of the most affected, not by chance during a chemotherapy cycle, each patient is subject to periodic withdrawals of blood samples to monitoring leukocyte trends. Lower levels in fact correspond to a functional decrease in the district with higher risk of infection and sepsis [4].

Furthermore, according to Elting, the same effects are observed according with Elting, chemotherapy-induced mucositis has an incidence ranging between 5% and 15% [5].

Chemotherapy is also associated with wide ranging adverse effects on nontarget tissues, including substantial impacts on the immune system [6]. Literature shows conflicting data from which it is impossible to find a clear answer about the behavior of the lymphocyte population after the end of treatment.

Neutropenia is often named as the most serious hematological toxicity and may involve infections that inevitably lengthen treatment time and would force the oncologist to a reduction in chemotherapy dosing [7-9].

B and T lymphocytes undergo major changes [10].

B lymphocytes are the precursors of antibody-forming cells and participate in humoral responses. T lymphocytes participate in delayed hypersensitivity reactions, rejecting foreign tissue grafts and tumors, eliminating virus-infected cells. In addition, they can amplify (T helper cells) or suppress (T suppressor cells) the reactions of B lymphocytes [11].

These cells are characterized by their ability to recognize a variety of antigen by their different antigen receptors and to generate the immunologic memory [12].

Knowledge of how immune mechanisms and inflammatory responses are regulated is critical for the understanding of the pathogenesis of complex diseases, such as periodontitis, which is an inflammation of the supporting tissues of the teeth with progressive attachment loss and bone destruction [13].

This study aims to assess changes in the immune system of patients suffering from different types of tumor and treated with different chemotherapies. For this reason, before and after chemotherapy treatment, immunohistochemical analyses of gingival biopsies of these patients, have been Conducted.

Materials and Method

Study design

Twelve patients were identified among 2500 cases of malignant carcinoma, lymphoma or leukemia at the UOC Hematology and Transplant Center PO "San Salvatore" in Pesaro and UOC Oncology and Dentistry PO "Santa Croce" Fano from January 2014 to May 2016. None of the patients have received chemo- or radiotherapy before tissue collection and none were affected by chronic periodontitis [14]. Only patient "A" was smoker.

The twelve patients selected needed chemotherapy treatment and dental treatment and were subjected to periodic prophylactic oral hygiene sessions.

The histopathological features of the tumor specimens were classified in accordance to WHO criteria [15].

Patients' clinicopathological data and molecules, dosage, mode of administration and duration of chemotherapy protocols are shown in detail in Table 1.

Group	Case	Age	Clinical diagnosis	Smoker	Chemotherapy	Time of administration	Placque
1	A	54	Follicular lymphoma	YES	Rituximab	1 cycle/2 months x 2 years	Absent
	I	38	High grade lymphoma	NO	R CHOP	1 cycle/21 days x 6 times	Absent
	L	70	High grade B lymphoma	NO	R CHOP	1 cycle/21 days x 6 times	Absent
2	B	62	Squamous cell carcinoma oral cavity	NO	Cisplatin	1 cycle/ week x 8 times	Absent
3	C	74	Chronic linfatic leukemia	NO	R Bendamustine	1 cycle/4 weeks x 6 times	Inflammation
4	D	67	Low Differentiated carcinoma	NO	Doxorubicine Cyclophospamide	1 cycle/3 weeks x 4 times	Absent
5	E	60	Invasive rectal carcinoma	NO	Folfox	1 cycle/2 weeks x 4 times	Absent
	H	70	Colic carcinoma	NO	Folfox	1 cycles/2 weeks x 12 times	Absent
6	F	88	Prostatic carcinoma	NO	Docetaxel	1 cycles/2 weeks x 6 times	Absent
	G	71	Ovaric heteroplasia	NO	Carboplatin Docetaxel	2 cycles/2 weeks x 6 times	Absent

Table 1: Characteristics of study participants from whom gingiva samples are collected.

The study protocol was approved by Human Etic review committee of Regione Marche with determine n. 59 of January 23, 2014 (CE13173) and 542 of July 31, 2014 (CE14090) and signed, in-formed consent was obtained from each patient. The written informed consent of all patients who participated in the experimental investigation described in this manuscript was obtained according to the Declaration of Helsinki, and for the nature of the procedure, possible discomforts and risks had been fully explained. Institutional Review Board approval was obtained for our study.

Gingival biopsy

Each gingival biopsy was planned only once plaque control was found to be<10% based on the O'lealry plaque index [16].

Each gingival biopsy was performed before chemotherapy start (baseline) protocol and after its end. For patient A we performed 3 gingival biopsies: before starting chemotherapy, after 3 dose of Rituximab and after its end.

The gingiva is the part of masticatory mucosa that covers the alveolar process and surrounds the cervical portion of the teeth. Gingiva is divided in marginal and attached ones and the coronal gingiva ending with the free gingival margin, which has a scalloped edge [17].

Apical gingiva continues with the alveolar mucosa that is mobile and darker red color; among them there is generally a well recognizable boundary line, called mucogingival junction.

After applying local anesthetic based on articaine hydrochloride with epinephrine 1:100000 tests were carried out in the palatal or lingual region. Mesial, distal or paramarginal releasing incisions where performed in gingiva around the tooth for withdrawal of the marginal portion.

The gingival biopsy included masticatory keratinized and nonkeratinized stratified squamous epithelium with connective portion (lamina propria).

Immunochemistry

Tissue samples were fixed in 10% formalin for 24 h at room temperature and embedded in paraffin. Morphologic analysis was performed on 4- to 6-µm-thick tissue sections stained with hematoxylin and eosin. Immunostainings were performed on conventional 5-µm-thick paraffin tissue sections on positively-charged slides. After heat drying, sections were dewaxed in xylene and rehydrated through a graded series of ethanol. To better unmask antigenic sites, two antigen retrieval solutions were applied, by incubating sections for 20 min in a Dako PT-Link autostainer (DakoCytomation): I) with a Dako Target Retrieval solution (DakoCytomation, Milano, Italy), pH 6.0, at 750 KW for 20 min before staining with anti-CD20 and CD117; II) with a Dako Target Retrieval solution (DakoCytomation), pH 9.0, at 750 KW for 20 min before staining with anti-CD3, -CD4, and –CD8, -CD1a, -CD56 and –S100.

Endogenous peroxidase activity was quenched incubating the sections in 3% (v/v) hydrogen peroxide for 7 min at room temperature. Tissue sections were then incubated with the following mono-clonal antibodies for 30 min: CD20 (clone L26, dil. 1: 200, DakoCytomation), CD3 (clone F7.2.38, dil. 1: 100, DakoCytomation), CD4 (clone 4B12, dil. 1: 20; Thermo Scientific, Fremont, CA, USA), CD8 (clone 1A5, dil. 1: 40; Novocastra Laboratories Ltd., Newcastle, UK), CD56 (clone 123C3, dil. 1: 100, DakoCytomation), CD1a (clone 010, dil. ready to use, DakoCytomation), CD117 (clone c-kit, dil. 1:500, DakoCytomation) and S100 (rabbit polyclonal, dil. 1: 400, DakoCytomation). The antigen-antibody complex was subsequently visualized using the Envision / HRP Detection System kit peroxidase/DAB (DakoCytomation). Sections were counterstained with Mayer's Hematoxylin (Bio-Optica SPA, Milano, Italy) and coverslipped with Paramount. Negative control slides omitting the primary antibody were included in all assays. For CD20, CD3, CD4, and CD8, we used tissue sections from a normal palatine tonsil as positive controls; whereas for CD56, CD1a, CD117 and S100 we used the fetal kidney tissue sections [18].

We counted the number of immunopositive cells using images of the histologic sections captured with the same digital system utilized for the histomorphometric analysis. Immunostained cells were enumerated in 10 representative and consecutive microscopic high-power fields (40X-HPF).

Representative hematoxylin and eosin-stained slides and immunostained slides were scanned using the APERIO ScanScope Digital System (Nikon, Firenze, Italy) and representative images were recorded using ImageScope software (Nikon) at the original magnification of 10X and 15X.

The immunohistochemical assessments were evaluated by two independent pathologists.

Inconsistencies were discussed until an agreement was reached.

Statistical analysis

All statistical analyses were performed using Microsoft Excel (Version 2016).

Descriptive statistics were developed using mean and standard deviation (SD) for quantitative data. The statistical unit of analysis was the patient, and all the analyses were performed at patient level, dividing values observed in either dermal or epithelium.

Results

Participants

Twelve patients were involved in the study between January 2014 to March 2016. Two patients dropped out.

Given the variability of the therapies administered to the patients involved, it was considered worthwhile to analyse them singularly.

Patient "A"

Patient "A" was administered with Rituximab with the following frequency: 1 cycle every two months for 2 years. The 54-years-old patient was a smoker and was affected by Follicular Lymphoma. His oral hygiene was in a good state. Three gingival biopsies were implemented every six months. Data collected are shown in Figure 1. The cellular population CD20 decreased at a dermal level from 5 to 1 cell per HPF. At a dermal level the indicators are stable between 1 and 0.The cellular population CD3 passed at an epithelial level from 10 cells to 2 in the first biopsy post-chemotherapy to stabilize at 18 per HPF in the last. The CD8 population increased both at a dermal and epithelial level passing respectively from 13 to 44 and 5 to 26 cells per HPF. The CD4 increased 4 times at a dermal level.

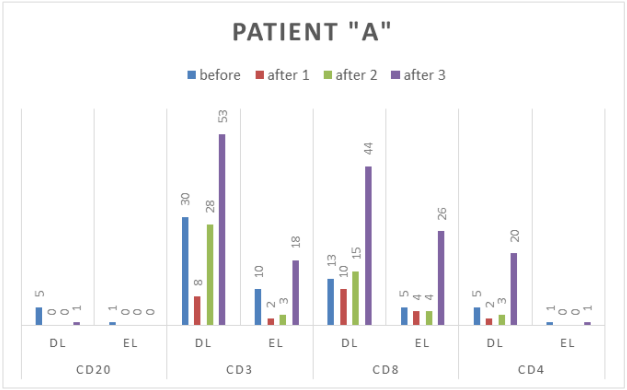

Figure 1: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "A". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

Patient "I"

Patient "I" was included in the R-CHOP protocol, which provides with a total of 6 cycles every 21 days. Although the diagnosis of high grade lymphoma, the odontostomatological situation was good. Immunohistochemical evaluation is shown in Figure 2. From the immunohistochemistry analysis, all the markers used were subjected to a decrease in number at a dermal level, while no variations were observed at an epithelial level; the CD20 passed from 6 to 0, the CD3

from 35 to 27 and the CD8 from 25 to 15, and finally the CD4, from 18 to 5.

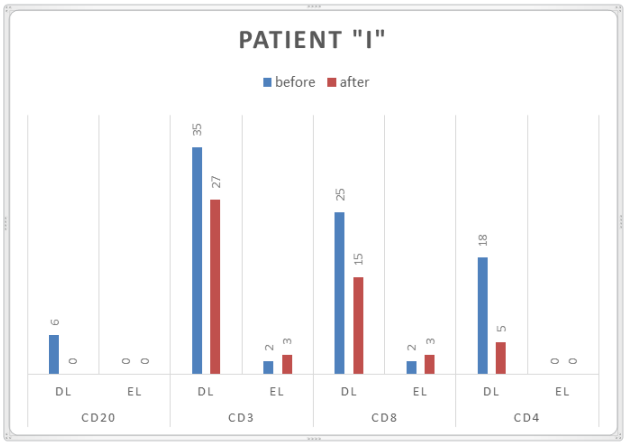

Figure 2: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "I". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

Patient "L"

Patient "L" was included in the same protocol R-CHOP administered to patient "I".This patient was diagnosed with a high grade lymphoma B. He was smoker, and the plaque was absent during the first visit. Although the most important variations are in the dermal set, we can find the same trend in the values of the epithelium (Figure 3).

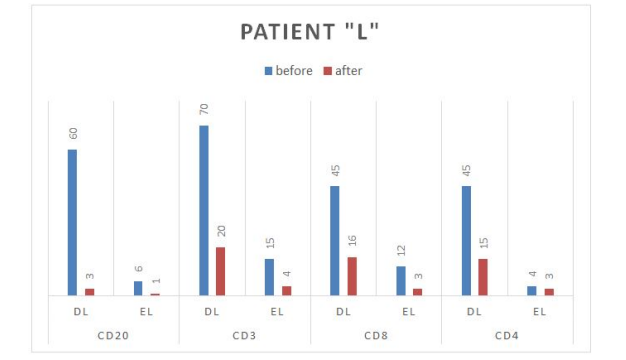

Figure 3: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "L". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

The count of the CD20 decreases from 60 to 3 cells per HPF in the derma and 6 to 1 in the epithelium.The same trend can be noticed in the CD3, which pass from 70 to 20 and 15 to 4 respectively in the derma and epithelium. Likewise, the CD8 decreased from 45 to 16 in the derma and from 12 to 3 in the epithelium. Similarly to the previous results, the number of CD4 passes from 45 to 15 in the derma and from 4 to 3 in the epithelium.

Patient "B"

Patient "B", who was diagnosed with an Oral squamous cell carcinoma, has been administered with 8 cycles of cisplatin, on a weekly deadline. From the immunohistochemistry analysis we infer that, apart the CD20 population, all the other show an increase in the values in the post-chemotherapy biopsy (Figure 4).

If the values increase in the derma at a maximum of 5 units at a epithelial level they increase up to four times with the epithelial CD3.

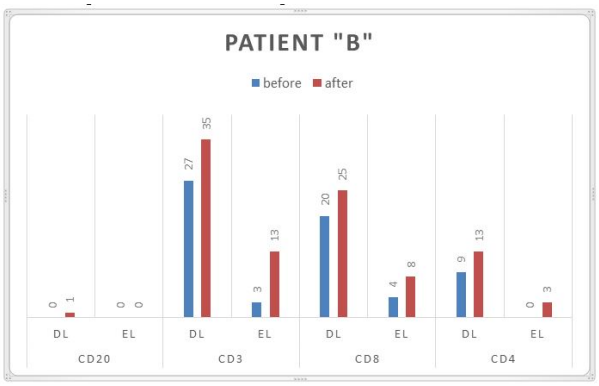

Figure 4: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "B". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

Patient "C"

Patient "C", age 74, was affected by chronic lymphocytic leukaemia, non-smoker, but at the moment of the first biopsy spread gingivitis symptoms were observed.

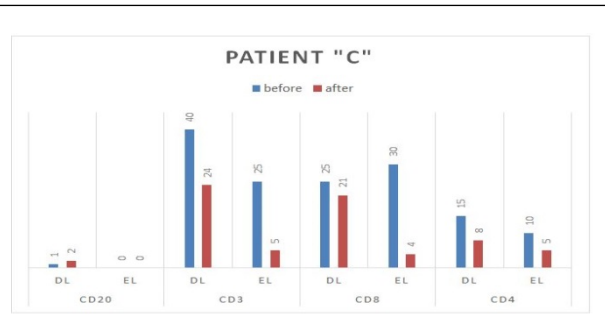

Figure 5: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "C". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

This patient was administered with R-Bendamustine for 6 cycles every 4 weeks. The values resulted decreased after the chemotherapy treatment (Figure 5).

CD20 data were stable, at a dermal level they passed from 1 to 2 cells per HPF and they were absent at the epithelial level. The CD3 set is shown to be decreased in the post-chemotherapy biopsy from 40 to 24 in the derma and from 25 a 5 in the epithelium.

CD8 population, as prior results, appeared decreased: from 25 to 21 in the derma, from 30 to 4 at an epithelial level.

We observed the same trend in CD4: at a dermal and epithelial level they were halved from 15 to 8 and from 10 to 5 per HPF respectively.

Patient "D"

Patient "D", positive for low differentiated carcinoma, was administered with Doxorubicine-Ciclofosfamide for 4 cycles (three times a week). At the moment of the first visit oral plaque was absent. Data collected are shown in Figure 6.

CD20 resulted increased at a dermal level from 60 to 100 cells per HPF, while were absent at an epithelial level. Differently from the previous results, the CD3 are stable with 100 cells per HPF in the derma and they decrease from 25 to 11 in the epitelium.

CD8 population is halved: 100 to 50 and from 10 to 4 respectively at a dermal and epithelial level.

Moreover, CD4 decrease from 100 to 70 in the derma and from 8 to 2 cells per HPF in the epithelium.

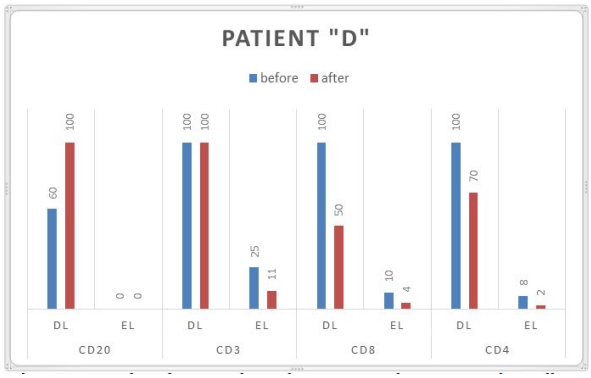

Figure 6: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "D". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

Patient "E"

Patient "E" was administered with protocol Folfox for 4 cycles, twice a week. The 60-year-old was a non-smoker, with a good level of oral hygiene and diagnosed with an invasive rectal carcinoma. The values show large drop (Figures 7 and 8).

The CD20 pass from 80 to 3 and from 5 to 1 in derma and epithelium. CD3 show the same trend: at a dermal level, the rate shows 100 at the first visit and 35 after the chemotherapy cycles, and at an epithelial level 30 and 10.

CD8 and CD4 perform a drop: the first two become a quarter of the initial value (100) in the derma, and nearly half at an epithelial level (from 12 to 7), whereas CD4 decreases from 110 to 16 and from 22 to 6.

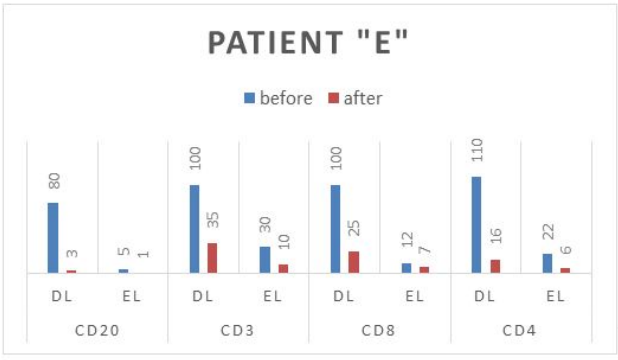

Figure 7: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "E". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

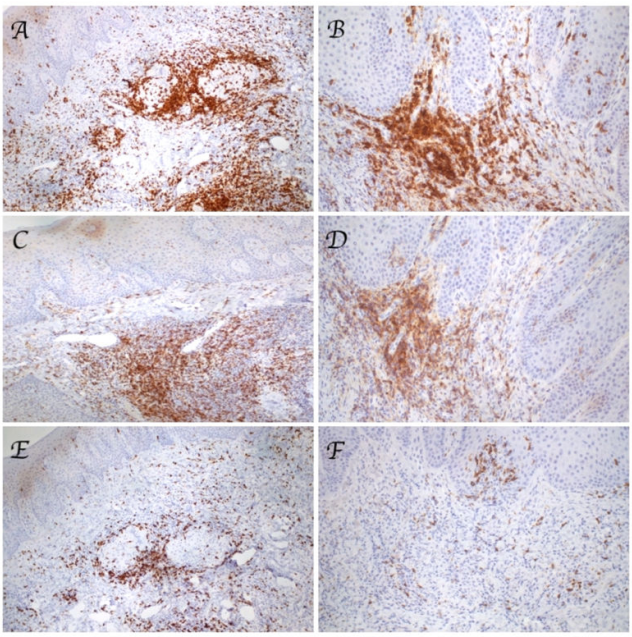

Figure 8: Case "E" Immunohistochemical detection of different lymphocyte subsets in epithelium and dermal tissue before and after chemotherapy: A and B) CD3+ cells; C and D) CD4+ cells; E and F) CD8+ cells. Magnifications at 40X.

Patient "H"

Patient "H", age 70, positive for colic carcinoma, as the previous patient, was administered with Folfox cycles, twice a week for 12 times. Oral hygiene level was good at the moment of the visit.

Data collected are shown in Figure 9.

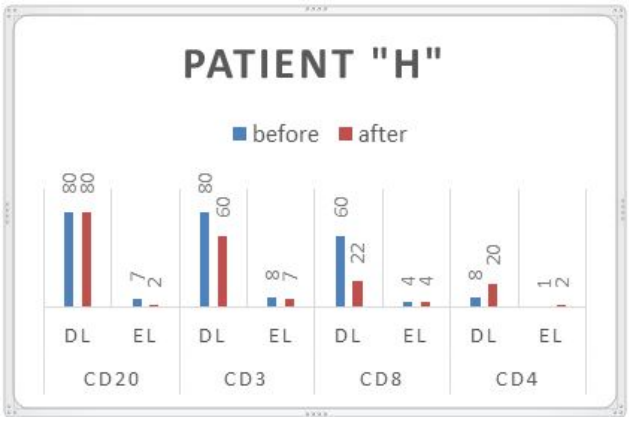

Figure 9: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "H". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

CD20 set remained constant at 80 cells per HPF at a dermal level, while it decreased from 7 to 2 at an epithelial level. CD3 vary from 80 to 60, in the derma and from 8 to 7 in the epithelium.

CD8 follow the same trend: the values are constant at an epithelial level, while at a dermal level they decrease from 60 to 22.

On the contrary, CD4 show an increase from 8 to 20 at an epithelial level.

Patient "F"

Patient "F", diagnosed with prostate cancer, was administered with 6 cycles of Docetaxel, twice a week.

Data observed results increased (Figures 10 and 11).

CD20 population increases from 0 to 20 at a dermal level and from 0 to 1 at an epithelial level.

The same trend can be observed in CD3, which increase from 12 to 80 in derma and from 13 to 25 in epithelium.

CD8 also show an increase: in the derma from 6 to 40 and in the epithelium form 12 to 26. CD4 increase respectively in derma and epithelium from 8 to 50 and from 5 to 4.

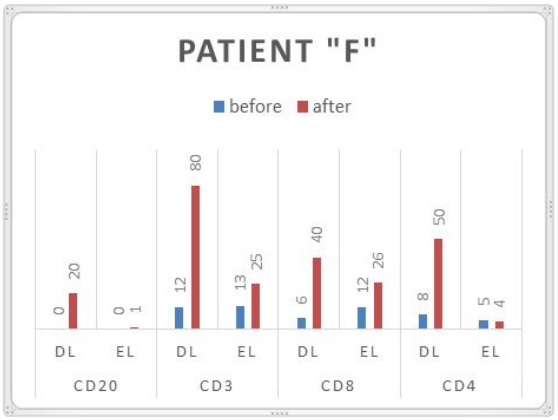

Figure 10: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "F". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

Patient "G"

Patient "G", diagnosed with ovaric heteroplasia, was administered with 6 weekly cycles of carboplatin-docetaxel. No oral plaque was observed during the first visit.

Results collected are described in Figure 12.

The rate of the CD20 population showed a decrease in the derma from (12 to 2) and unvaried in the epithelium (null).

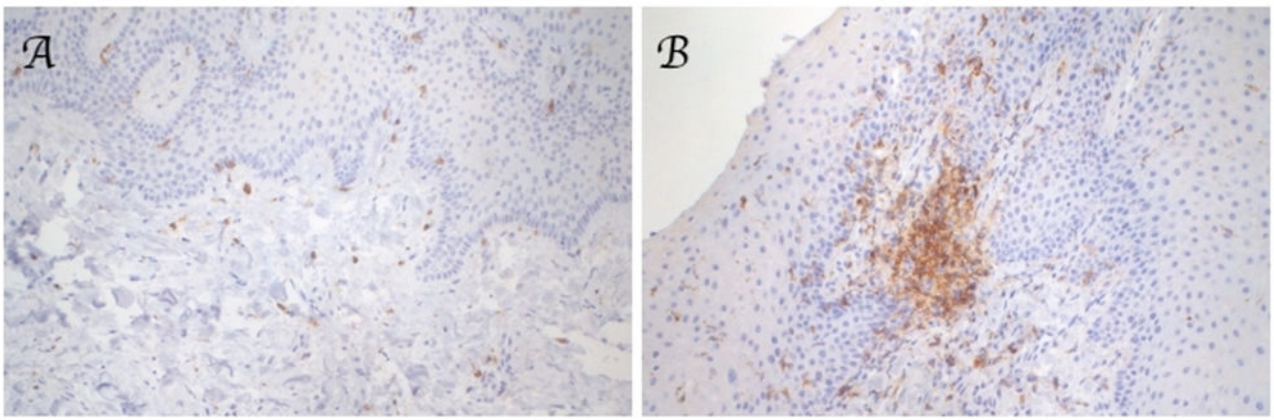

Figure 11: Case "F" Immunohistochemical detection of CD4+ lymphocyte subsets in epithelium and dermal tissue before (A) and after (B) chemotherapy. Magnifications at 40X.

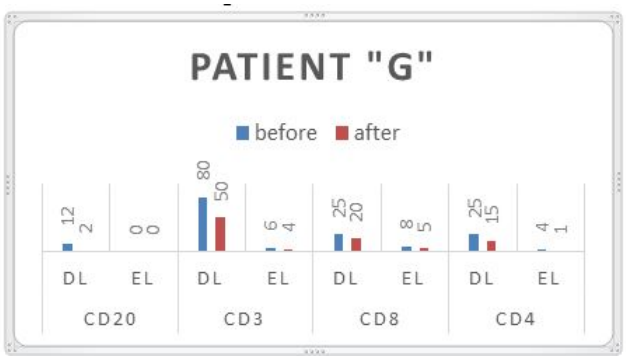

Figure 12: Results of immunohistochemistry analysis in samples collected before chemotherapy (Before) and after chemotherapy (After) in patient "G". DL=Dermal Level; EL=Epithelial Level. Numbers are referred like cells for high power field (HPF).

CD3 performed a decrease both in derma and epithelium (from 80 to 50 and from 6 to 4).

Related values to CD8 and CD4 show a decrease, at a dermal level, they vary from 25 to 20 and from 8 to 5 respectively, while at an epithelial level, they vary from 25 to 15 and from 4 to 1.

Discussion

Data collected describe numerical alterations of markers analyzed after chemotherapy administration in periodontal tissues of diseased patients.

The obtained results reflect the same effects described in literature in peripheral circulation of patients treated administering chemotherapy.

Rituximab specifically binds to the transmembrane antigen, CD20+, expressed in mature B cells and normal or neoplastic pre-B including more than 95% of all non-Hodgkin lymphomas in B (NHL cells), thereby blocking the proliferation pathological [18]. Rituximab, recognizing targeting B cells, induces a complete depletion in the peripheral circulation [19] that shows also an effect in the periodontal tissues.

Platinum-based drugs inactivate STAT6 protein, causing a decrease in the levels of PD-L2 protein associated with the action of CD4+ CD25+ regulatory T lymphocytes (Treg) [20-22].

Cisplatin has been shown to have the same indirect stimulatory action even when given in combination with gemcitabine [23].

Bendamustine has been shown to cause lymphopenia affecting T cell population and if associated with Rituximab as in our study, also B cell [24].

Studies report an incidence of grade 3 or 4 lymphopenia in 62% of cases [25]. The percentage rises in combination with solid tumors up to 91%-92% [26-27].

Cyclophosphamide is a very reactive compound which can interact not only with the DNA, but also with RNA and proteins. These events can therefore stop cell division and cause chromosomal aberrations. The combination of cyclophosphamide, doxorubicin can aggravate myelosuppression.

Cyclophosphamide exerts contradictory effects on lymphocytes, metronomic administration regimen selectively depletes Treg in human while preserving other lymphocyte subsets in number and function. Surprisingly, this regimen does not inhibit but on the contrary, it dramatically enhances T and NK cell functions through its suppressive effect on Treg number and function. The author observes that in peripheral blood Treg lymphocytes suffer a large decrease in both percentage and absolute terms (7.9 before *vs.* 3.1 after; 28.7 cells/mm^3 before *vs.* 6.4 cells/mm^3 after). This numerical change is described in all 9 patients in the study [28].

Regarding Folfox applications the effects caused in the peripheral circulation are not yet clarified. Literature describes a decrease in circulating myeloid-derived suppressor cells (MDSCs) after Folfox treatment with increased expression of the CD247 protein, a key molecule that regulates immune function and regulates the activity of T lymphocytes and NK cells [29].

Docetaxel has been demonstrated to exert both immunomodulatory and immunosuppressive action [30].

After administration of Docetaxel Treg decrease on the total circulating CD4+ lymphocytes number, CD4+ Teffs (effectors)/CD4+ Treg cells ratio increases by about 20% (average value) as shown by Roselli [4]. Docetaxel is well associated to severe lymphopenia both T (CD4+ and CD8+) and NK (CD56+) cells not dose dependent [31].

These results are consistent with studies demonstrating variations of the same markers in peripheral circulating blood in patients treated using chemotherapeutic drugs. Only in patient "F", treated using Docetaxel, results obtained are discordant with decrease described by previous study [31] but no data available describes clearly an immune system enhancement.

In fact, Docetaxel is well associated to not dose dependent severe lymphopenia both T (CD4+ and CD8+) and NK (CD56+) cells [31].

With regards to all markers considered, in patients treated using Rituximab, R-Chop, Rituximab-Bendamustine, Doxorubicine-Cyclophosphamide, Folfox and Carboplatin-Docetaxel we observed an upward trend, while we noticed a downward trend in those treated administering Cisplatin and Docetaxel.

Data collected can be considered as part of effects of drug administered.

Chemotherapeutics drugs can be subdivided by their mechanism of action in [10]:

1) alkylating agents, which crosslink DNA strains, cyclophosphamide; 2) antimetabolites agents that interfere with synthesis of DNA and RNA; 3) topoisomerase inhibitors which prevent the correct unwinding of DNA; 4) antibiotics like bleomycin which favours overgerations of reactive oxigen species; 5) microtubular poisons like paclixatel that prevents polymerization and depolymerization of tubulin; 6) immune checkpoint blockers ICBs like nivolumab, targeted on PD1 receptors [32].

Several recent studies show that modern regimens cause changes to the immune system that linger long after the end of treatment.

CD20+ B lymphocytes and CD4+ T lymphocytes levels remain depleted also nine months after the end of therapy [6]. Verma observed that after 2 weeks after the end of the regimen all four subtypes of lymphocytes (B, CD4+ T, CD8+ T, NK) have decreases in numbers compared to the "pre-chemotherapy levels". B cells have become 5.4%

of the initial value. After 9 months, CD8+ T lymphocytes and NK cells have totally similar numerical values to the initial ones. Only CD20+ B lymphocytes and CD4+ T lymphocytes showed reduced values, reaching respectively 69% and 60% of initial levels.

Chemotherapy-induced myelosuppression frequently results in the succession of opportunistic pathogens at oral mucosal sites, including the periodontium, with subsequent local or systemic infection [33].

Several studies describe a shift from gram-positive to gram negative in periodontally diseased sites in cancer patients [34]; chemotherapeutic regimens "promote" the entry of pathogens of red complex [35] in the deep pockets [36] that reach the bloodstream and cause systemic infections [37].

The signs of periodontitis can be related to immune cells and their products involved in the response, as shown by Yoshie et al. [38], implicating activated T lymphocytes in periodontal disease pathogenesis. However, data were also accumulating in support of B cell involvement in bone resorption, using multiple myeloma as a model [39].

One of the parameters influenced by the number of B and T lymphocytes is alveolar bone level. Activated T and B cells can be the cellular source of RANKL in bone resorption process in periodontal disease gingival tissue [40]. If activated by specific antigens, T cells act as producers of Rankl [41] and thus become able to directly activate osteoclast genesis. Surprisingly bone loss due to periodontal disease in immunocompetent rats is greater than in immunosuppressed mice [42].

The same research group on a mouse model observed that the average loss of bone in the absence of T cells and two of their cytokines, interleukin-6 (IL-6) and interferon-gamma (IFG) is reduced by 70% when compared to the value measured in immunocompetence situation (0.08 mm *vs* 0.28 mm) [43].

Overall, human CD4+ T cells, are identified as essential mediators of alveolar bone destruction [44].

It has been shown that in deficiency of B cells during infection by *P. Gingivalis* alveolar bone loss is lower than in presence of B cells probably due to lack of production of IgG anti *P. Gingivalis* [45]. B lymphocytes, especially B1 subclass, also appear to act positively on osteoclast genesis through the expression of RANKL [46,47].

B cells also play a direct role in bone formation, probably due to their interaction with cells and molecules specifically involved in the process [48,49], they are responsible for 45% of the total bone marrow production of osteoprotegerin [50].

Although *in vitro* HIV-infected rats show reduced bone loss, clinically it has been observed that HIV-positive patients with an increased progression of periodontitis. Statistically we were not able to find differences in the incidences between seropositive and seronegative [51]. This happens because in the absence of CD4+ lymphocytes B other cells can produce bone-resorptive cytokines.

Moreover in transplanted patients taking immunosuppressant drugs several authors observed bone loss but it was supposed that it was part of side effects and not caused by lymphocyte depletion [52,53].

In conclusion, with our pilot study, we try to describe changes happening during chemotherapy on lymphocytes population of periodontal tissues.

The study, considering the limitations of the samples considered, offers potential ideas to draw more precise protocols, designated on the presented pilot data. No data about hystomorphometric parameters are yet available.

Statistical analysis conducted cannot provide detailed information because of little sample considered. Standard deviation cannot be taken in consideration. Further studies will be conducted to compare our findings on chemotherapy and periodontal reaction.

This should result in a better understanding of the clinical significance of immune system cells in diagnosis and, consequently, in the selection of the suitable therapeutic opportunities to prevent development of periodontitis in patients receiving chemotherapy.

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Conflict of Interest Statement

The author declares that there is no conflict of interest in this study.

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