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MARIA ALICE GATTI PALMA

Fatores relacionados ao paciente e ao implante, associados ao perfil inflamatório do fluído crevicular peri-implantar de implantes instalados em pacientes com histórico de periodontite agressiva e crônica

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Orientador: Prof. Dr. Márcio Zaffalon Casati

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Resumo

O objetivo deste estudo de coorte foi determinar os fatores relacionados ao paciente e ao implante, associados à liberação de citocinas no fluído crevicular peri-implantar (PICF), durante a cicatrização inicial e o processo de osseointegração de implantes instalados em pacientes com histórico de doença periodontal agressiva e crônica. Para isso, por meio de envelopes pardos, foi aleatorizada a instalação de noventa e dois implantes bone level ou tissue level, em pacientes parcialmente edêntulos, apresentando histórico de doença periodontal agressiva ou crônica, ou saúde periodontal. Aos 15 e 60 dias após a instalação dos implantes, foi realizada a coleta do PICF para avaliação dos níveis de IL-1β, TNF-α, IL-6, IL-8, IFN-γ, GM-CSF, IL-4, IL-10, IL-12 e IL-13, por meio da tecnologia Luminex/Magpix. Os fatores relacionados ao paciente e ao implante foram: gênero, idade, condição periodontal (saúde, periodontite agressiva ou periodontite crônica), região de instalação do implante (anterior ou posterior), torque de inserção (≤15, 15-35, ou ≥35N), profundidade de sondagem peri-implantar, sangramento à sondagem, presença ou ausência de biofilme, análise da frequência de ressonância (ISQ), tipo de plataforma do implante (bone ou tissue level), comprimento do implante (8, 10 ou 12 mm), diâmetro do implante (3.3, 4.1 ou 4.8 mm), e diâmetro da plataforma do implante (3.3, 3.5, 4.1, 4.8, ou 6.5 mm). Os dados obtidos foram avaliados por regressão logística múltipla, considerando um nível de significância de 5%. A análise estatística indicou que aos 15 dias, a liberação de citocinas no PICF está associada ao gênero (GM-CSF), posicionamento do implante na arcada (IFN-γ, IL-4 e IL-8), torque de inserção (IFN-γ), sangramento à sondagem (IFN-γ), tipo de plataforma do implante (IFN-γ), histórico de periodontite (IL-1β e IL-6), e presença de biofilme (IL-8). Após 60 dias da cirurgia de colocação do implante, a liberação de citocinas esteve associada à idade (GM-CSF, IL-6, IL-12 e IL-13), diâmetro da plataforma do implante (IFN-γ), profundidade de sondagem (IL-10), presença de biofilme (IL-6 e IL-10), sangramento à sondagem (IL-12), posicionamento do implante na arcada (IL-1β), diâmetro do implante (IL-1β) e torque de inserção (IL-8). O comprimento do implante e a análise de frequência de ressonância não estiveram associadas à liberação das citocinas nos períodos avaliados. Deste modo, os resultados deste estudo sugerem que diferentes características relacionadas ao paciente e ao implante podem influenciar o conteúdo do fluído crevicular peri-implantar, durante a cicatrização inicial e o processo de

osseointegração, em pacientes com histórico de doença periodontal agressiva e crônica.

Palavras-chave: Implantes dentais. Periodontite agressiva. Periodontite Crônica. Cicatrização. Osseointegração. Citocinas.

Abstract

The aim of this cohort study was to determine the implant and patient-related factors, associated to the release of peri-implant crevicular fluid (PICF), during early healing and osseointegration process of implants placed in patients with history of aggressive and chronic periodontal disease. For this, through brown envelopes, it was randomized the insertion of ninety-two bone level or tissue level implants, in patients with history of aggressive or chronic periodontitis, or periodontal health. At 15 and 60 days after implants insertion, PICF was collected to assessment the levels of IL-1β, TNF-α, IL-6, IL-8, IFN-γ, GM-CSF, IL-4, IL-10, IL-12 and IL-13, using Luminex/Magpix assay. The implant and patient-related factor were: gender, age, periodontal condition (health, aggressive periodontitis, and chronic periodontitis), region of implant insertion (anterior or posterior), insertion torque (≤15, 15-35, or ≥35N), peri-implant probing depth, bleeding on probing, presence or absence of biofilm, resonance frequency analysis (ISQ), type of implant platform (bone or tissue level), length of implant (8, 10 or 12 mm), diameter of implant (3.3, 4.1 or 4.8 mm), and diameter of implant platform (3.3, 3.5, 4.1, 4.8, or 6.5 mm). The data were assessed using multiple logistic regression analysis, with a significance level of 5%. The statistical analysis indicates that at 15 days cytokines release were influenced by gender (GM-CSF), position of implant in the arch (IFN-γ, IL-4, IL-8), insertion torque (IFN-γ), bleeding on probing (IFN-γ), type of implant platform (IFN-γ), periodontal condition (IL-1β, IL-6), and biofilm (IL-8). At 60 days cytokines release were influenced by age (GM-CSF, IL-6, IL-12, IL-13), diameter of implant platform (IFN-γ), probing depth (IL-10), biofilm (IL-6, IL-10), bleeding on probing (IL-12), position of implant in the arch (IL-1β), implant diameter (IL-1β) and insertion torque (IL-8). The length of implant and the resonance frequency analysis were factors not associated to the release of cytokines, in all follow-up periods. Thus, these results support that different implant and patient-related characteristics could influence the PICF composition during early healing and osseointegration process of dental implants placed in patients with a history of aggressive and chronic periodontitis.

Keywords: Dental implants. Aggressive periodontitis. Chronic periodontitis. Wound healing. Osseointegration. Cytokines.

Sumário

1 INTRODUÇÃO

O aumento do número de implantes dentais instalados para reposição de dentes perdidos por doença periodontal fez com que crescessem os questionamentos sobre as possibilidades destes pacientes apresentarem maiores riscos de complicações. Isto porque, apesar das altas taxas de sobrevivência dos implantes dentais usados na reabilitação de rebordos parcial ou totalmente edentulos já terem sido claramente mostradas em diversos estudos (Schou *et al*., 2006; Karoussis *et al*., 2007; Chambrone *et al*., 2013; Chambrone *et al*., 2014a; Chambrone *et al*., 2014b), quando se avalia os critérios de sucesso, os pacientes com histórico de doença periodontal apresentam maiores profundidades de sondagem e perda óssea peri-implantar, além de uma incidência mais alta de peri-implantite, quando comparados à pacientes com saúde periodontal (Schou *et al*., 2006; Karoussis *et al*., 2007; Ong *et al*., 2008; Van der Weijden *et al*., 2005; Matarasso *et al*., 2010; Monje *et al*., 2014). Assim, pacientes com histórico de periodontite apresentam taxas de sucesso variando de 52.4% a 100, enquanto pacientes sem histórico de periodontite apresentam taxas de sucesso que variam de 79.1% - 100% (Brocard et al., 2000; Karoussis et al., 2003; Rosenberg et al., 2004; Mengel & Flores-de-Jacoby, 2005).

Renvert *et al*., (2009), sugeriram que a doença periodontal existente ou prévia, é capaz de influenciar a inflamação peri-implantar, em especial a doença periodontal agressiva, na qual os indivíduos apresentam uma resposta imunológica alterada frente aos patógenos orais (Armitage *et al*., 1999). Com relação à doença peri-implantar, a presença de biofilme é fundamental, entretanto, os microrganismos isoladamente não são capazes de provocar destruição tecidual. É necessária uma complexa interação entre as bactérias e a subsequente resposta imunológica do paciente susceptível para que os tecidos peri-implantares sejam afetados, semelhantemente ao que ocorre na doença periodontal.

Nesse sentido, as citocinas e outros marcadores inflamatórios presentes no fluído crevicular peri-implantar (PICF) podem refletir de maneira acurada o status inflamatório dos tecidos peri-implantares, correlacionar-se com as condições clínicas, e predizer a destruição peri-implantar futura (Kaklamanos *et al*., 2002; Reinhardt *et al*., 2010; Petkovic *et al*., 2010). Monitorar o PICF destes pacientes imunologicamente suscetíveis se tornou mais importante desde que estudos prévios mostraram que a mucosa peri-implantar aparentemente saudável, pode ser naturalmente caracterizada

por um estado inflamatório subclínico (Nowzari *et al*., 2008; Emecen-Huja *et al*., 2013). Esta condição pró-inflamatória da mucosa peri-implantar foi demonstrada durante o processo de cicatrização inicial em regiões que haviam recebido a cirurgia de colocação de implante, e apresentavam dente adjacente (Emecen-Huja *et al*., 2013). Emecen-Huja *et al*. (2013), relataram que uma semana após a cirurgia, os implantes apresentaram maiores níveis de IL-6, IL-8, MIP-1β e TIMP-1 no fluído crevicular peri-implantar (PICF) quando comparados aos sítios dos dentes adjacentes à cirurgia. No mesmo sentido, Nowzari *et al*. (2008), ao comparar o fluído crevicular de dentes e implantes saudáveis, encontraram quase o dobro de concentração de TNF-α e lL-8 ao redor dos implantes.

Estes resultados, além de indicarem que a mucosa peri-implantar apresenta um perfil pró-inflamatório mesmo em condições clínicas aparentemente saudáveis, mostram a utilidade da análise das citocinas do PICF em detectar os primeiros sinais de inflamação. Esta ferramenta se torna ainda mais relevante, uma vez que os parâmetros clínicos comumente registrados para avaliar o estado da saúde peri-implantar não são capazes de proporcionar estre diagnóstico.

Além disso, deve-se considerar que alguns fatores como a idade, frequência de escovação diária, intervalos de controle de placa profissional, profundidade de sondagem e índice de placa podem influenciar a liberação de citocinas no PICF, como demonstrado por Recker *et al*. (2015). Indicando que fatores sistêmicos e locais podem influenciar a composição do PICF, contudo, podem existir muitos outros capazes de influenciar a composição do PICF, que ainda não foram avaliados. Determinar quais são e a magnitude de sua influência são fundamentais para conhecimento e controle do que ocorre no ambiente peri-implantar tanto no momento da cicatrização inicial, quanto ao longo do tempo. Dessa forma, o presente estudo incluiu uma ampla análise de possíveis fatores relacionados aos implantes e aos pacientes que possam estar associados à liberação de citocinas no PICF, e sobre os quais não há relato na literatura.

Adicionalmente, a integração dos implantes dentais nos tecidos mole e duro representa o resultado de uma complexa cascata de eventos biológicos que se inicia com a intervenção cirúrgica. Após a osteotomia e inserção do implante no osso alveolar, ocorre a formação de coágulo, e a partir deste, inicia-se a maturação óssea em contato com a superfície de titânio. Do ponto de vista biológico, a resposta imune-inflamatória desencadeada com a instalação dos implantes (Ivanovski *et al*., 2011),

envolvendo estágios inflamatórios, angiogênicos e osteogênicos, conduz ao estabelecimento da osseointegração (Salvi *et al*., 2015). Esses eventos são coordenados pela liberação de uma série de moléculas sinalizadoras, entre as quais estão as citocinas, quimiocinas, e fatores de crescimento (Nguyen *et al*., 2009; Midwood *et al*., 2004; Salvi *et al*., 2015).

Contudo, embora uma resposta imune-inflamatória e seus desdobramentos moleculares sejam esperados, e de fato devam ser considerados necessários para modular as fases iniciais da cicatrização, seus papéis no processo de osteogênese e cicatrização após a instalação dos implantes não são totalmente compreendidos (Salvi *et al*., 2015). Diante de todas essas proposições que permanecem indefinidas, uma melhor compreensão da resposta imune-inflamatória durante os períodos de cicatrização inicial e osseointegração auxilia na sugestão de como será o desenvolvimento das condições clínicas dos tecidos moles e duros, além de sugerir novos alvos para estratégias com o objetivo de aprimorar a performance clínica dos implantes dentais (Kolar *et al.*, 2010; Salvi *et al.*, 2015).

Assim, neste estudo foi investigada a hipótese de que características relacionadas ao paciente e ao implante podem estar associadas à liberação de citocinas no fluído crevicular peri-implantar, durante o processo de cicatrização inicial e osseointegração.

2 ARTIGO: Patient and implant-related factors associated to inflammatory profile in peri-implant crevicular fluid of implants placed in patients with history of aggressive and chronic periodontitis.

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Abstract

OBJECTIVES: The aim of this cohort study was to determine the implant and patient-related factors, associated to the release of peri-implant crevicular fluid (PICF), during early healing and osseointegration process of implants placed in patients with history of aggressive and chronic periodontal disease.

MATERIALS AND METHODS: Through brown envelopes, it was randomized the insertion of ninety-two bone level or tissue level implants, in patients with history of aggressive or chronic periodontitis, or periodontal health. At 15 and 60 days after implants insertion, PICF was collected to assessment the levels of IL-1β, TNF-α, IL-6, IL-8, IFN-γ, GM-CSF, IL-4, IL-10, IL-12 and IL-13, using Luminex/Magpix assay. The implant and patient-related factor were: gender, age, periodontal condition (health, aggressive periodontitis, and chronic periodontitis), region of implant insertion (anterior or posterior), insertion torque (≤15, 15-35, or ≥35N), peri-implant probing depth, bleeding on probing, presence or absence of biofilm, resonance frequency analysis (ISQ), type of implant platform (bone or tissue level), length of implant (8, 10 or 12 mm), diameter of implant (3.3, 4.1 or 4.8 mm), and diameter of implant platform (3.3, 3.5, 4.1, 4.8, or 6.5 mm). The data were assessed using multiple logistic regression analysis, with a significance level of 5%.

RESULTS: The statistical analysis indicates that at 15 days cytokines release were influenced by gender (GM-CSF), position of implant in the arch (IFN-γ, IL-4, IL-8), insertion torque (IFN-γ), bleeding on probing (IFN-γ), type of implant platform (IFN-γ), periodontal condition (IL-1β, IL-6), and biofilm (IL-8). At 60 days cytokines release were influenced by age (GM-CSF, IL-6, IL-12, IL-13), diameter of implant platform (IFN-γ), probing depth (IL-10), biofilm (IL-6, IL-10), bleeding on probing (IL-12), position of implant in the arch (IL-1β), implant diameter (IL-1β) and insertion torque (IL-8). The length of implant and the resonance frequency analysis were factors not associated to the release of cytokines, in all follow-up periods.

CONCLUSIONS: These results support that different implant and patient-related characteristics could influence the PICF composition during early healing and osseointegration process of dental implants placed in patients with a history of aggressive and chronic periodontitis.

KEYWORDS: dental implants; aggressive periodontitis; chronic periodontitis; wound healing; osseointegration; cytokines.

Introduction

Patients with history of periodontal disease, especially aggressive periodontitis, have a lower survival and success rate of dental implants than periodontally health individuals (Mengel *et al*., 2007; Ong *et al*., 2008; Levin *et al*., 2011; Swierkot *et al*., 2012; Wen *et al*., 2014; Monje *et al*., 2014; Sousa *et al*., 2015; Zangrando *et al*., 2015). This phenomenon may be explained by an imbalance between the release of pro and anti-inflammatory cytokines in response to bacterial challenge that usually occurs in these kinds of patients (Teles *et al*., 2010; Donos *et al*., 2012). This becomes even more important in the consideration of dental implant rehabilitation, once previous studies have indicated that even in apparent healthy implants at early healing and in function, a higher pro-inflammatory status could be noted (Nowzari *et al*., 2008; Emecen-Huja *et al*., 2013).

It is worth mentioning that the clinical parameters commonly used to assess peri-implant status are limited in detecting early changes. However, recent studies pointed out the utility of peri-implant crevicular fluid (PICF) analysis as an approach to detect early alterations in tissue homeostasis and inflammatory status (Kaklamanos *et al*., 2002; Emecen-Huja *et al*.,2013). For example, IL-1β and TNF-α were found in higher levels in sites of peri-implant mucositis, peri-implantitis and also in sites during implant osseointegration, than in healthy implants (Petkovic *et al*., 2010; Fonseca *et al*., 2014). Interestingly, recent study showed that PICF profile could be modified by some systemic and local characteristics, as peri-implant probing depth and presence of biofilm (Recker *et al*., 2015). However, a number of other variables associated to implant design, history of periodontal disease (aggressive or chronic), tissue around the implant, insertion torque and others may also influence the release profile of cytokines in PICF, although, up to date, there is no study confirming these aspects, and this is the first study focused on this assessment.

In the present study, we hypothesized that some implant and patient characteristics have an impact in the wound healing process of peri-implant tissues of patients with history of aggressive and chronic periodontitis. Thus, the purpose of this

study was to assess the levels of cytokines in the peri-implant crevicular fluid in patients with history of chronic and aggressive periodontitis, and determine factors related to patients (local and systemic) and to implants that could influence the release of cytokines assessed, at early stage of implant wound healing and after osseointegration process.

Materials and Methods

Population screening and study design

This cohort study was designed in accordance with the STROBE statement and followed the standards of Ethics Committee of Piracicaba Dental School (017/2010), which all participants signed the informed consent form.

A hundred one partially edentulous patients were recruited from the Graduate Clinic of Piracicaba Dental School, University of Campinas, between March 2011 and November 2013. The eligibility criteria were: (1) patients ≥ 18 years; (2) single missing tooth with adjacent teeth present; (3) diagnosis of health or history of generalized chronic periodontitis (GCP) or generalized aggressive periodontitis (GAgP) (Armitage, 1999), previously treated by own group of periodontists, with at least 1 year of supportive periodontal therapy (SPT); (4) a full-mouth plaque score (FMPS) (Ainamo & Bay 1975) and full-mouth bleeding score (FMBS) (Muhlemann & Son 1971) <20%; (5) signed the informed consent form.

The exclusion criteria were as follows: (1) presence of systemic diseases (e.g., diabetes) that possibly affect the healing process; (2) smokers and former smokers; (3) antibiotic therapy within 6 months prior to implant placement; (4) pregnancy or lactating; (5) the absence of keratinized tissue at the implant site; (6) necessity of bone or soft-tissue graft; (7) untreated periodontitis; and (8) unwillingness to comply with procedures and follow-up visits. And the exit criteria were: (1) voluntary withdrawal; (2) non-compliance with study procedures or visits; (3) development of systemic or oral diseases requiring antibiotic or anti-inflammatory therapy; and (4) development of peri-implant infection/alteration requiring surgical intervention.

Aggressive and chronic periodontitis were initially treated in previous studies (Casarin *et al*., 2012; do Vale *et al*., 2015), or in the Graduate Clinic of the

Piracicaba Dental School. All patients received mechanical debridement at least one year before implant surgery, however, in the aggressive periodontitis cases was associated systemic antibiotics (amoxicillin and metronidazole) or local antimicrobial (Povidone iodine). All them were kept in maintenance care program and received scaling and root planning in the sites presenting probing depth (PD) ≥ 5mm and bleeding on probing (BOP) during this supportive therapy). None of the periodontitis patients presented sites with PD ≥ 5mm at the implant surgery.

Following screening/recruitment visit and eligibility verification, surgery was scheduled. Immediately prior to surgery, full-mouth clinical parameters were recorded. The peri-implant crevicular fluid were obtained at 15 and 60 days after surgery. At 30 days of follow-up, peri-implant clinical parameters were assessed. And, at 60 days full-mouth and implant clinical parameters were also recorded.

Surgical and post-operative protocol

Cone beam computerized tomography, models and diagnostic wax-up were used to implant planning. Anti-inflammatory therapy consisting of 4 mg dexamethasone, 1h before surgery was applied. Before surgery, each patient's oral cavity was rinsed with 0.12% chlorhexidine for 1 minute. Following local anesthesia, a midcrestal incision was made, and full-thickness buccal and palatal/lingual mucoperiosteal flaps were reflected. After full-thickness flap elevation, osteotomy site was prepared using custom-made surgical template. The implants selected for this study were tissue level (standard plus) or bone level type with sandblasted acid-etched surfaces (Straumann® AG, Basel, Switzerland). Each patient received one screw and for the random allocation were used brown sealed envelopes containing one label (tissue level or bone level). The envelopes were opened only at the end of drills sequence and were discarded after all. The implants were inserted according to a standard one-stage surgical protocol following the manufacturer's recommendations and using the manufacturer-specified surgical drills. During implant placement, insertion torque which could be <15N, within 15-35N, and >35N was measured using the Straumann ratchet (Straumann® AG, Basel, Switzerland). A healing abutment was inserted (standard one-stage protocol), and soft tissues were sutured with interrupted nylon sutures (Ethicon, Johnson & Johnson do Brasil, SP, Brazil). Post-operative

instructions included 1-week abstinence from mechanical biofilm control in the surgical sites, antimicrobial rinse with 0.12% chlorhexidine (Periogard, Colgate-Palmolive Brasil, SP, Brazil), twice a day for 7 days, and analgesic if patients felt pain or discomfort. Sutures were removed 7 days after surgery, and patients were instructed to resume their usual mechanical oral hygiene.

Clinical Parameters

The clinical parameters were performed by a single calibrate examiner (interclass correlation of 90% for PD), by means of a manual probe (PCPUNC 15, HuFriedy, Chicago, IL, USA), guided by an acrylic stent, and were recorded six regions per tooth/implant (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual). The full-mouth assessments were performed before and 60 days after implant placement surgery. The peri-implant clinical parameters were obtained at 30 and 60 days after surgery. The clinical parameters assessed were: (1) plaque index (PI) for teeth (Ainamo and Bay, 1975) and plaque index modified by Mombelli *et al*.(1987) for implants; (2) bleeding on probing (BOP) for teeth (Mühlemann and Son, 1971) and modified bleeding on probing index for implants (Mombelli *et al*., 1987); (3) probing/peri-implant sulcus depth (PD); (4) relative gingival/mucosa margin position (rGMP), the distance from the gingival/mucosa margin to the stent margin; and (5) relative clinical attachment level (rCAL), the distance from the bottom of the pocket/peri-implant sulcus to the stent margin.

After implant placement, implant stability was assessed by resonance frequency analysis (RFA). Implant stability was again assessed at 60 days after implant placement. The analysis was performed following the manufacturer's guidelines: a transducer (implant system / diameter specific; Smartpeg^{TM}, Straumann USA, LLC) was hand-torqued into implant body to measure implant stability by RFA with Osstell ISQ device (Osstell, Gothenburg, Sweden). The values were recorded three times at mesial face and three at distal.

Peri-implant Crevicular Fluid Collection

Peri-implant crevicular fluid samples were collected at 15 and 60 days after implant surgery. The supragingival biofilm was removed, implants were washed and the area was isolated (with cotton rolls) and gently dried, then, peri-implant crevicular fluid (PICF) was collected from mesial and distal sites of each implant. PICF was collected by the insertion of paper strips (2 strips per site) (Periopaper, Oraflow, Plainview, NY, USA) into the peri-implant sulci until a slight resistance. The strips were maintained in place for 15s. The fluid volume was measured with a calibrated electronic device (Periotron 8000; Oraflow), and the strips were placed in sterile tubes containing 400 µL phosphate-buffered saline (PBS) with 0.05% Tween (Sigma Aldrich, St.Louis, MO, USA). The papers contaminated with blood and saliva were discarded and the fluid collection repeated.

Sample Preparation and Analysis

Cytokine levels of granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13 and tumor necrosis factor (TNF)-α in PICF were determined with the high-sensitivity human cytokine 10-plex (Millipore Corporation, Billerica, MA, USA). Assays were carried out according to the manufacturer's recommendations with the MAGPIX® instrument (MiraiBio, Alameda, CA, USA). The samples were individually analyzed (each implant separately), and mean concentration of each marker was calculated based on the individual as a statistical unit and expressed as pg/mL.

Statistical Analysis

The analyses were performed by a blinded biostatistician who did not know the patient's status before the results. Only data from patients complying with all the evaluations were used in the statistical analysis. The numeric variables clinical parameters for teeth (PD, rCAL, rGMP, PI and BOP), clinical parameter for implants (PD, rCAL, rGMP), RFA and patient age, were initially evaluated by the Shapiro-Wilk test (for normality). Those presenting a Shapiro-Wilk p-value > 0.05 were analyzed by repeated measures ANOVA followed by Tukey's test. Those presenting a Shapiro-Wilk p-value ≤ 0.05 were analyzed by the Friedman test (intragroup comparisons) and

Kruskal-Wallis/Dunn tests (intergroup comparisons). Comparisons among groups in terms of distribution of gender, position of implant in the arch, type of implant platform, frequency of implant insertion torque, and the peri-implant clinical parameters PI and BOP, were performed by χ^2 test.

The concentration of cytokines at 15 and 60 days, separately, were dichotomized by the median and it represented the dependent variables being analyzed. The independent variables evaluated were the patient and implant-related factors: gender (female and male), periodontal status (healthy, aggressive periodontitis or chronic periodontitis), age (dichotomized by the median into ≤ 44 years and > 44 years), position of implant in the arch, position of implant in the arch (anterior or posterior), insertion torque torque (≤15, 15-35, or ≥35N), peri-implant probing depth (≤2.5mm, 2.5-3mm, >3mm), bleeding on probing (dichotomized by the median into ≤24.9% and >24.9%), biofilm (presence or absence), type of implant platform (bone or tissue level), implant length (8, 10 or 12 mm), diameter of implant (3.3, 4.1 or 4.8 mm), diameter of implant platform (3.3, 3.5, 4.1, 4.8, or 6.5 mm), and resonance frequency analysis (dichotomized by the median into ≤73.2 and >73.2 for 15 days, and ≤80 and >80 for 60 days).

The dependent variables were analyzed for normality using the Shapiro-Wilk test. Non-normal distributions were observed for all tested cytokines, so they were dichotomized at the median (≤median or >median), and the logistic regression was performed. A univariate analysis (χ^2 test) was performed ($p \leq 0.2$) to select the variables for the multiple regression model. For the final multiple regression model, all variables with a p-value less than 0.2 were included. Moreover, to compare cytokine levels between the factors associated with their release a Mann-Whitney or Kruskal-Wallis/Dunn test were used. All tests had the significance level fixed at 5% and were performed using SPSS 21 (IBM SPSS Statistics, Armonk, NY).

Results

Study population and clinical parameters

Ninety-two patients (43.86±9.77 years old; 69.5% females) completed the study. Patient exclusion reasons were: failure to comply with the protocol, missing one

or more appointments (n=4) (GAgP patients: 01 bone level and 01 tissue level; GCP patients: 01 bone level and 01 tissue level); post-operative infection complications requiring additional treatment (n=3) (Healthy patients: 02 bone level and 01 tissue level), and early implant loss (n=2) (GCP patients: 01 tissue level; Healthy patients: 01 bone level), as shown in Figure 1.

Subject's demographics and clinical status are shown in Table 1. Aggressive periodontitis patients were younger (33.60±4.11 years old) than chronic periodontitis (49.09±6.30 years old) and healthy patients (45.78±10.52 years old) ($p<0.05$). No significant differences were observed among groups in terms of distribution of gender ($p>0.05$). Healthy patients presented lower values of clinical parameters such as full-mouth PD, rCAL, and rGMP when compared with aggressive and chronic periodontitis patients at baseline and 60 days of follow-up. No significant differences were observed among groups as regards to the initial and final PI and BOP ($p>0.05$). Aggressive periodontitis patients had more cases of implant rehabilitation in the anterior region than the others groups ($p<0.05$). No differences among groups were noted regarding to implants distribution of type of implant platform and insertion torque ($p>0.05$). No differences among groups were noted for RFA, however, there was an increase in ISQ values at 60 days after surgery. Regarding peri-implant parameters (Table 2) (PD, rCAL, rGMP, PI and BOP) statistical analysis indicated no differences ($p>0.05$) among all groups at baseline and 60 days of follow up.

Biological Parameters – Cytokines Evaluation at 15 days

Table 3 shows the results of the univariate analysis, and table 4 presents the patient and implant variables associated with release of inflammatory markers in PICF at 15 days after implant surgery after multivariate logistic regression. The univariate analysis showed that IL-10 was associated with gender (p=0.183), length of implant (p=0.197), diameter of implant (p=0.158), insertion torque (p=0.110), and biofilm (p=0.103). IL-12 was associated with gender (p=0.135), diameter of implant platform (p=0.086), and insertion torque (p=0.048). IL-13 was associated with gender (p=0.135), length of implant (p=0.184), diameter of implant platform (p=0.062), and history of periodontal disease (p=0.103). And, TNF-α was associated with age (p=0.112), and probing depth (p=0.155). However, after the construction of multiple

modeling, these associations were not maintained, and were not considered how risk factors.

The association between GM-CSF and gender at univariate analysis (p=0.023) was maintained after multiple modeling. Male patients were most protected to have this cytokine on PICF (OD 0.107, p=0.047).

At χ^2 test, IFN-γ was associated with: position of implant in the arch (p=0.005), type of implant platform (p=0.115), diameter of implant platform (p=0.039), insertion torque (p=0.011), history of periodontitis (p=0.023), and bleeding on probing (p=0.039). When multiple regression was applied, diameter of implant platform and history of periodontitis were excluded. The final model demonstrated that the implants inserted at posterior region presented 5.143 more times the risk of release IFN-γ (OR 5.143; p= 0.010) than the anterior region. Higher torques are more protected against release of IFN-γ than low torques (OR 0.106, p=0.003 for 15-35N; OR 0.72, p=0.002 for >35N). Implants with bleeding on probing >24% presented four more times the risk release IFN-γ (OR 4.357, P=0.007) than implants with BP ≤24,9%. The implants tissue level showed more protection against the presence of IFN-γ on PICF than bone level (OD 0.279, p=0.016).

The χ^2 test showed an association between IL-1β and history of periodontitis (p=0.072). After construction of multiple modeling, this association was maintained and exhibited two more times risk of chronic periodontitis patients release this cytokine (OR 2.779, p=0.056), and the aggressive periodontitis patients three more times (OR 3.375, p=0.039).

At univariate analysis IL-4 was associated with position of implant in the arch (p=0.007), diameter of implant (p=0.108), and insertion torque (p=0.096), however these last two factors did not affect the final p value at multiple modeling, so, they were removed. The final model showed that the implants inserted at posterior region presented four more times the risk to release IL-4 on PICF than the anterior region (OR 4.303, p=0.01).

The univariate analysis indicated an association among IL-6 and position of implant in the arch (p=0.024), diameter of implant (p=0.023), diameter of implant platform (p=0.041), history of periodontitis (p=0.048), and biofilm (p=0.029). However, the final model included only the history of periodontitis, and showed three more times

risk of patients with history of chronic periodontitis presented IL-6 in PICF (OR 3.077, p=0.033).

At χ^2 test, IL-8 was associated with position of implant in the arch (p=0.024), diameter of implant (p=0.023), diameter of implant platform (p=0.041), and biofilm (p=0.029). When multiple regression was applied, the variables diameter of implant and diameter of implant platform were excluded, and showed that the implants at posterior region had three more times risk to have IL-8 in PICF (OR 3.149, p=0.039), and the presence of biofilm had two more times this risk (OR 2.904, p=0.044).

Biological Parameters – Cytokines Evaluation at 60 days

Table 5 shows the results of the univariate analysis, and table 6 presents the patient and implant variables associated with release of inflammatory markers in PICF at 60 days after implant surgery after multivariate logistic regression. The univariate analysis showed that IL-4 was associated with gender (p=0.161), position of implant in the arch (p=0.080), diameter of implant platform (p= 0.026), insertion torque (p= 0.160), probing depth (p=0.178), bleeding on probing (p=0.189) and biofilm (p= 0.127). The TNF-α was associated with bleeding on probing (p=0.080). For these cytokines, However, after the construction of multiple modeling, regression analyses showed no statistical association with any independent variables. Thus, no multiple regression analysis models were constructed.

The risk indicators are shown at Table 6. For GM-CSF levels, the χ^2 test presented associations with age (p=0.044), insertion torque (p=0.109), and probing depth (p=0.089). When multiple regression was applied, insertion torque and probing depth was excluded because they negatively affected the overall importance of the model, and only age presented a significant effect on GM-CSF levels. Patients >44 years old are more protected against GM-CSF than youngers (OR 0.277, p=0.048).

For IFN-γ levels, from the univariate analysis position of implant in the arch (p=0.080), diameter of implant platform (p=0.010), insertion torque (p=0.060), probing depth (p=0.075), bleeding on probing (p=0.189), and biofilm (p=0.127) were selected for a regression model. The final model for IFN-γ showed an association with diameter of implant platform. The diameter 4.1mm presented nine more times to release IFN-γ

than 3.3mm (OR 9.42, p=0.015) and the 4.8mm nineteen more times (OR 19.5, p=0.018). The *p* value for 3.5 and 6.5mm were not significant (p=0.116 and 0.762, respectively).

For IL-10, at χ^2 test, associations were determined with age (p=0.055), probing depth (p=0.024) and biofilm (p=0.042). In multiple logistic analyses only probing depth and biofilm remained statistically significant. Implants with probing between 2,5-3 mm are more protected from this cytokine than shallower probing (OR 0.218, p=0.004). The p value for probing depth >3mm was not significant (p=0.675). The presence of biofilm presented three more times to have IL-10 in PICF than the absence (OR 3.893, p=0.019).

The univariate analysis indicated association of IL-12 with age (p=0.036), implant length (p=0.175), insertion torque (p=0.009), and bleeding on probing (p=0.024). At multiple logistic analyses, implant length and insertion torque were excluded, and bleeding on probing, and age remained statistically significant. Implants with >24% of BP presented more protection against IL-12 (OR 0.123, p=0.022) than implants with ≤24%, as well as older patients are more protected for this cytokine (OR 0.194, p=0.025).

For IL-13 levels, the univariate analysis showed associations with age (p=0.044), implant diameter (p=0.070), and history of periodontitis (p=0.116). After multiple logistic regression analyses only age remained significant. Older patients are more protected from IL-13 (OR 0.277, p=0.048).

For IL-1β levels, the χ^2 test indicated associations with position of implant in the arch (p=0.024), implant diameter (p=0.189), and biofilm (p=0.127), and all these were included in multiple model. In the final model, position of implant in the arch and implant diameter remained statistically significant. Implants at posterior region have fourteen more times to have IL-1β in PICF than the anterior region (OR 14.201, p=0.015). Implants with diameter 4.1mm are more protected against IL-1β than the 3.3mm (OR 0.097, p=0.031). The p value of diameter 4.8mm was not significant.

The IL-6 levels were associated with gender (p=0.206), age (p=0.001), history of periodontitis (p=0.123) and biofilm (p=0.055). When multiple logistic regression analysis was applied, gender and history of aggressive were excluded because they negatively affected the overall importance of the model, and only age

and biofilm presented a significant effect on IL-6 levels. Older patients are more protected to have IL-6 in PICF than youngers (OR 0.197, p=0.001). And implants with presence of biofilm have three more times to release this cytokine (OR 3.376, p=0.04).

For IL-8, at univariate analysis there were associations with gender (p=0.161), position of implant in the arch (p=0.080), type of implant platform (p=0.200), and insertion torque (p=0.056), and these were included in multiple model. In the final model, only insertion torque (OR 0.247, p=0.021) remained statistically significant. Implants with insertion torque between 15-35N are more protective from IL-8 than these <15N. The p value of torques >35N was not significant.

Discussion

In the present study we hypothesized that the release of cytokines in PICF at early healing and during osseointegration process, in patients with history of aggressive and chronic periodontitis, could be influenced by characteristics related to patient and implant. In fact, cytokine profile was influenced by gender (GM-CSF), age (GM-CSF, IL-12, IL-13, IL-6 and IL-8), implant position in the arch (IFN-γ, lL-4, IL-6, IL-8 and IL-1β), type of implant platform (IFN-γ), implant diameter (IL-1β), diameter of implant platform (IFN-γ), insertion torque (IFN-γ and IL-8), history of periodontitis (IL-1β and IL-6), and, clinical parameters (peri-implant sulcus depth (IL-10), biofilm (IL-8, IL-6 and IL-10), and bleeding on probing (IFN-γ and lL-12). Length of implant and the RFA did not influence the release of cytokines.

There is a suggestion amongst most of the studies that peri-implant tissue is characterized by a pro-inflammatory status, even in clinical health conditions (Nowzari *et al*., 2008; Nowzari *et al*., 2012; Recker *et al*., 2015). This pro-inflammatory profile in implants was also observed during wound healing (from surgery to 12 weeks), with a more pronounced release of pro-inflammatory cytokines at peri-implant sites, when compared to teeth sites (Emecen-Huja *et al*., 2013). However, the assessment of cytokines profile and the factors that influence their release have not been evaluated during early stage and osseointegration process in patients characterized by a higher pro-inflammatory response, like patients with a history of aggressive and chronic periodontitis. This knowledge could bring light to understand which characteristic could predict a more pro-inflammatory environment around implants, which might be

associated with a higher rate of biological complications found in these patients, such as increased probing depth and bone loss.

At 15 days after implant placement, history of periodontitis was a relevant factor associated with the release of IL-1β and IL-6 (Table 4), since GCP patients exhibited two more times risk to release IL-1β (OR 2.779, p=0.056), and the GAgP patients have three more times this risk (OR 3.375, p=0.039). And, GCP patients showed three more times risk of presented IL-6 in PICF (OR 3.077, p=0.033). IL-1β and IL-6 are potent pro-inflammatory cytokine linked to inflammatory cell migration, stimulation of leukocytes and resident cells to produce other inflammatory mediators, and osteoclastogenesis process (Graves, 2008; Fonseca *et al*., 2009). In addition to promote bone resorption, these cytokines also interfere in bone formation process, through inhibiting osteogenic differentiation (Behl *et al*., 2008; Moxhan *et al*., 1995; Kwan Tat *et al*., 2004; Ding *et al*., 2009; Lacey *et al*., 2009; Tomomatsu *et al*., 2009). These results suggest that early implant healing in aggressive and chronic periodontitis patients have a higher pro-inflammatory profile, when compared to patients without history of periodontitis. Indeed, this hyper-inflammatory response in GAgP and GCP has also been reported in diseased and healthy dental sites, with an imbalance in the release of pro and anti-inflammatory cytokines, probably due to a hyper-reactive phenotype of phagocytes found these patients (Duarte *et al*., 2009; Gustafsson *et al*., 2006; Shaddox *et al*., 2010; Teles *et al*., 2010).

The position of implant in the arch influenced the concentration of IFN-γ, IL-4 and IL-8 in the PICF (Table 4). Implants inserted at posterior region presented five more times the risk of release IFN-γ (OR 5.143; p= 0.010), four more times the risk to release IL-4 (OR 4.303, p=0.01), and three more times risk to release IL-8 (OR 3.149, p=0.039) in PICF than the implants at anterior region. IFN-γ activates phagocytes and promotes the production of inflammatory cytokine, like TNF-α, IL-1β and chemokines (Murphy & Reiner, 2002; Shroder *et al*., 2004; Appay *et al*., 2008; Sallusto & Lanzavecchia, 2009; Gao *et al.*, 2007; Garlet *et al.*, 2008). IL-8 is a potent chemotactic agent for neutrophils, involved in the acute inflammatory response, peri-implantitis, and at early post-operative responses to surgical trauma (Okada & Murakami, 1998; Emecen-Huja *et al*., 2013; Nowzari *et al*., 2008; Nowzari *et al*., 2012). The presence of IFN-γ and IL-8 in the posterior region suggest that early healing of this implants is characterized by an exacerbated inflammatory response than the anterior. Interestingly, there are evidences that implants placed in posterior region tend to

exhibit more biologic complications (Rodoni *et al*., 2005). Additionally, IL-4 was also associated to posterior region. IL-4 presents marked suppressive and anti-inflammatory properties, mediated by its capacity to inhibit the transcription of pro-inflammatory cytokines (Murphy & Reiner, 2002; Appay *et al.*, 2008; Sallusto & Lanzavecchia, 2009; Agnello *et al.*, 2003; Jarnicki & Fallon, 2003; Bluestone *et al.*, 2009). Thus, the presence of IL-4 in implants inserted at the posterior region may indicate an attempt to control the inflammatory process stimulated by pro-inflammatory cytokines. However, a specific explanation for the tendency of greater cytokine production observed in posterior region is not available. It should be considered that the differences in the anatomy, histology, and function could account for the expression of certain cytokines (Nowzari *et al*, 2012; Recker *et al*., 2015).

Besides the position of implant in the arch, the type of implant platform also influenced the IFN-γ levels (Table 4). The implants tissue level showed more protection against the presence of IFN-γ on PICF than bone level (OD 0.279, p=0.016). Previous reports showed that the level of the abutment connection, in respect to the bone crest, have an especially importance in determining the crestal alveolar bone loss around implants (Dursun *et al*., 2012). The subcrestal and the bone level position of implant may favor the colonization of anaerobic Gram-negative species close to bone crest, which may be involved in triggering a pro-inflammatory response, changing the profile of cytokine released during the early healing period (Nowzari *et al*., 2012; Kano *et al*., 2007).

Still at 15 days, IFN-γ level was associated with insertion torque (Table 4). Higher torques are more protected against release of IFN-γ than low torques (OR 0.106, p=0.003 for 15-35N; OR 0.72, p=0.002 for >35N). It is assumed that after implant placement with high insertion torque there is a pronounced bone remodeling at the interface implant-bone region, while, in implants inserted with lower torque there is a more rapid bone formation (Berglundh *et al*., 2003). In fact, the histological study of Duyck *et al*. (2015) reported a trend of new bone formation and a significant increase in bone implant contact over the healing time of 4 weeks in low insertion torque implants (< 10 Ncm). Although the higher risk of IFN-γ detection in low insertion torque implants may seem a contradictory result, it's important highlight the multifunction of this cytokine: studies *in vivo* demonstrates that IFN-γ presents a pro-inflammatory effect through up-regulation of TNF-α and IL-1β levels (Gao *et al*., 2007; Garlet *et al*.,

2008), however, on the other hand, studies *in vitro* demonstrated that IFN-γ inhibits the RANKL signaling via degradation of the RANK adapter protein TRAF6 by the ubiquitin-proteasome system, which could attenuate osteoclastogenic events (Takayanagi *et al*., 2000), favoring bone formation. Finally, the IFN-γ detection was associated with bleeding on probing (Table 4). Implants with bleeding on probing >24% presented four more times the risk to release IFN-γ (OR 4.357, P=0.007) than implants with BP ≤24,9%, this result might be expected, since IFN-γ also presents pro-inflammatory properties (Garlet *et al*., 2010).

Gender of the patient was relevant in the release of GM-CSF at 15 days (Table 4), once men were most protected to release GM-CSF than women (OD 0.107, p=0.047). GM-CSF is a pro-inflammatory mediator, which can have critical roles in chronic diseases with bone resorption, including *P. gingivalis*-induced periodontitis (Lam *et al*., 2015; Hamilton *et al*., 2008; Bismar *et al*., 1995). Interestingly some studies reported an influence of sexual dimorphism in immune-inflammatory function, showing a pro-inflammatory innate immune response in women (Van Eijk *et al*., 2007; Bain *et al*., 2009). However, the impact of GM-CSF in the peri-implant tissues is not yet fully understood, since literature investigating the influence of GM-CSF in dental implants is scarce. Therefore, more studies are necessary to analyze the role of GM-CSF over the time in the peri-implant tissues.

In addition to their role in wound healing after installation of the implants, inflammatory cytokines may also be involved in the process of osseointegration. In this sense, we conducted an evaluation of these inflammatory cytokines to 60 days after implant placement, period in which the implants used in this study become osseointegrated (Cochran et al., 2002). The patient's age was a relevant factor for the release of GM-CSF, IL-6, IL-12, and IL-13 (Table 6). Patients >44 years old are more protected against GM-CSF (OR 0.277, p=0.048), as well as IL-12 (OR 0.194, p=0.025), IL-13 (OR 0.277, p=0.048) and IL-6 (OR 0.197, p=0.001) in PICF than youngers. It's important highlight that patients with aggressive periodontitis were younger than those with chronic periodontitis or healthy, this information may influence the interpretation of results. Remarkably, the aging process results in a decrease in cell function derived by a gradual deficiency of the regenerative response of certain tissues (Sousounis *et al*., 2014). Furthermore, studies from models of infections has demonstrated a decrease in the capacity of older individuals to produce specific antibody (Frasca *et*

al., 2011), and alterations in T-cell activation profiles that could affect antibody levels (McArthur *et al*., 1996, Ebersole *et al*., 2008; Haynes & Swain, 2012). This becomes important after implant placement, because a number of resident and inflammatory cells are recruited during wound healing to release several biological mediators, like growth factors and pro and anti-inflammatory cytokines that will lead to tissue repair (Gurtener *et al*., 2008). Therefore, the altered release of cytokines in the PICF observed in this study, influenced by the age of patients, might affect the tissue repair after implant placement, suggesting a poor wound healing in older patients.

The biofilm influenced the release of the pro-inflammatory IL-6 and the anti-inflammatory IL-10 (Table 6). The presence of biofilm presented three more times to have IL-10 (OR 3.893, p=0.019), and three more times to release IL-6 (OR 3.376, p=0.04) in PICF than the absence of biofilm. Indeed, the biofilm is considered a risk factor to release pro-inflammatory cytokines (Renvert *et al*., 2015). Interestingly, the anti-inflammatory cytokine IL-10 is widely expressed in inflamed periodontal tissues, in which it is thought to be associated with lower disease severity (Garlet, 2010). Studies have suggested that IL-10 can act in multiple ways, promoting the suppression of innate immune cytokines, once IL-10 interferes directly with IFN-γ and IL-17 production by T-cells, and also modulating both MMPs and RANK systems (Garlet *et al*., 2010; Pestka *et al.*, 2004; Lappin *et al*., 2001; Garlet *et al.,* 2004; Garlet *et al*., 2006). Therefore, the release of IL-10 at sites with presence of biofilm could represent an attempt to counterbalance the pro-inflammatory and destructive effects of IL-6.

Furthermore, IL-10 was associated to probing depth (Table 6). Implants with probing between 2,5-3 mm are more protected from this cytokine than <2,5mm (OR 0.218, p=0.004). The p value for probing depth >3mm was not significant (p=0.675). This results are in accordance with previous studies that reported that IL-10 has a tendency to be higher in shallow sites compared to deep sites in dental implants (Fonseca *et al*., 2014), or in moderate than deep pocket in generalized aggressive periodontitis (Casarin *et al*., 2010). Moreover, Cosgarea *et al*. (2012) founded elevated levels of IL-10 at implants when compared with teeth after implant insertion, highlighting the importance of this cytokine in the healing phase at implant sites.

At 60 days after surgery, the diameter of platform influenced the IFN-γ release (Table 6). The diameter 4.1mm presented nine more times to release IFN-γ than 3.3mm (OR 9.42, p= 0.015) and the 4.8mm nineteen more times (OR 19.5,

p=0.018). The p value for 3.5 and 6.5mm were not significant (p=0.116 and 0.762, respectively). For this cytokine, there was a trend of increase its concentration with the increasing size of the implant platform. It is an important observation that the sample size of implants with platform 6.5 is limited (only 2 implants), therefore, going irrelevant in the general analysis.

Furthermore, implants at posterior region presents fourteen more times to have IL-1β (OR 14.201, p=0.015) in PICF than the anterior region (Table 6), suggesting that the imbalance in the release of inflammatory markers observed at early healing with high levels of IFN-γ and IL-8 remains at 60 days. As previously mentioned, IFN-γ promotes the production of inflammatory cytokine as IL-1β. So, the high levels of IL-1β founded in the posterior region in this period may be a result of the process initiated by the higher release of IFN-γ during the early healing. Moreover, once osseointegration was established at this phase, elevated levels of IL-1β may result in deleterious effects in the peri-implant bone. In this sense, Ozgur *et al*. (2015) in a long term follow up, reported a higher peri-implant marginal bone loss in posterior regions.

Another factor that influenced the release of cytokines in this period was the insertion torque (Table 6). Implants with insertion torque between 15-35N (OR 0.297, p=0.021) are more protective from IL-8 than these <15N. The p value of torques >35N was not significant. This result may be related to different ratios in bone formation and resorption, which occurs as a result of higher or lower insertion torques (as previously discussed). Besides being a major chemoattractant for neutrophils, IL-8 can also play a critical role in bone metabolism by providing signals for the trafficking of osteoblast and osteoclast precursors, their differentiation and activity (Sahinguer & Yeudall, 2015; Bendre *et al*., 2003; Souto *et al*., 2014).

The diameter of implant is associated with the IL-1β release (Table 6). Implants with diameter 4.1mm are more protected against IL-1β than the 3.3mm (OR 0.097, p=0.031). The p value of diameter 4.8mm was not significant. Lower levels of IL-1β may indicate a lower rate of bone remodeling in wider implants, when compared to narrower implants. Indeed, Jimbo *et al*. (2013) demonstrated by histomorphometric analysis more bone formation around narrower implants (3.75 mm) at 5 weeks of healing, when compared to implants with wider implants (5 mm). These results can be explained due to differences in the rates of bone remodeling during early stages of healing, while depends on numerous factor including implant macrogeometry and its

interplay with the surgical instrumentation dimensions, which can affect the bone structure and cell availability and viability at the drilled site (Jimbo *et al*., 2013). Furthermore, it has been reported that the diameter of the implant would significantly influence the strain levels and concentration in the crestal bone during insertion and under functional loading (Petrie & Williams 2005; Qian *et al*., 2009), leading to the development of different profiles in PICF.

The evolution of osseointegration process involves biological events which were modulated by cytokines. Clinically, without the support of PICF analysis, its suggest that this process can be monitored through the RFA obtained by Osstell (ISQ values) (Meredith *et al*., 1996). This study showed no differences of RFA among groups (Table 1), however, there were increase of ISQ values between baseline and 60 days of follow-up, this is assigned to the establishment of osseointegration. On the other hand, the regression analysis showed no association with the ISQ values and release of cytokines. So, despite the RFA monitor the implant stability, given by bone-implant contact, this tool does not reflect the biological pathways involved in this process.

Considering the results of the present study, it is evident that wound healing of peri-implant tissues were influenced by several characteristics related to patient and implant. After 15 days of implant surgery the implant bone level, posterior region of the arch, women, presence of BOP, biofilm, lower insertion torque and history of periodontitis are considered as critical factors for release of inflammatory cytokines in the PICF. While after 60 days, are critical the following factors: older patients, posterior region of the arch, narrow implants, wider platforms, presence of BOP, biofilm and lower insertion torque. Interestingly, presence of biofilm, bleeding on probing, posterior region of the arch and lower insertion torque were associated with a greater release of pro-inflammatory in both evaluations. Thus, these characteristics should be included in future studies to determine their impact in success and survival rates of implants placed in patients with history of aggressive and chronic periodontitis.

Conclusion

The results of this study indicate that several patients and implants characteristics modulates the release of the inflammatory mediators in the peri-implant crevicular fluid during early healing and after osseointegration process.

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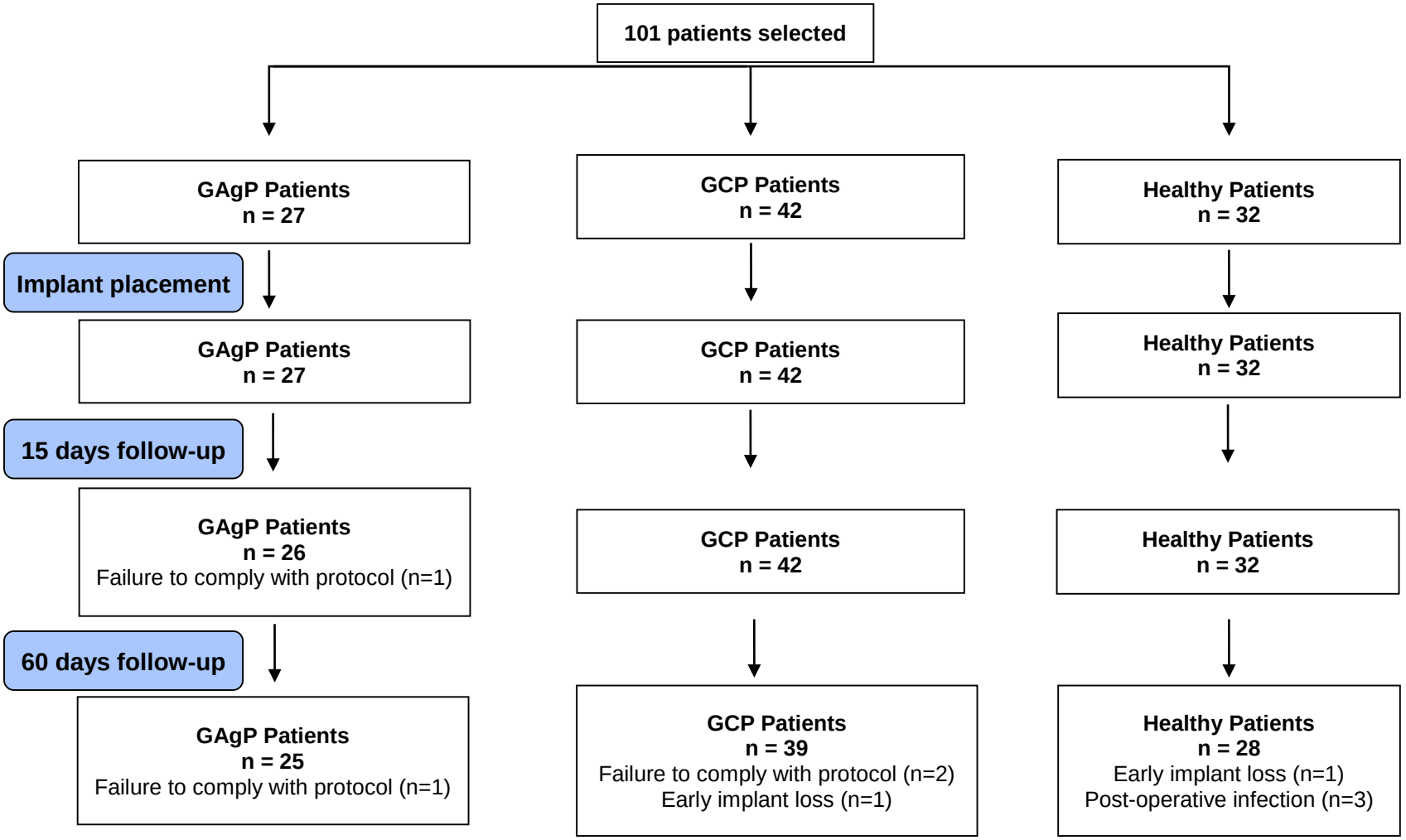
Figure 1. Flowchart of patients selected for study.



Abbreviations: GAgP, Aggressive periodontitis; GCP, chronic periodontitis.

Table 1. Study population demographic and clinical parameters for teeth, evaluated at baseline and 60 days after dental implant insertion.

Parameter	Aggressive Periodontitis	Chronic Periodontitis	Healthy
Age (yrs - mean ± SD)	33.60 ± 4.11 b	49.07 ± 6.30 a	45.78 ± 10.52 a
Gender - Woman - n (%)	23 (85.2%)	28 (71.80%)	15 (53.58%)
p-value	0.2988		
Full-mouth PD (mm - mean ± SD)			
Baseline	2.42 ± 0.28 Aa	2.32 ± 0.30 Aa	2.06 ± 0.29 Ab
60 days	2.43 ± 0.28 Aa	2.29 ± 0.26 Aa	2.06 ± 0.23 Ab
Full-mouth rCAL (mm - mean ± SD)			
Baseline	5.54 ± 0.99 Aa	5.48 ± 0.81 Aa	4.57 ± 0.69 Bb
60 days	5.69 ± 1.16 Aa	5.56 ± 0.77 Aa	4.64 ± 0.69 Ab
Full-mouth rGMP (mm - mean ± SD)			
Baseline	3.12 ± 0.82 Aa	3.16 ± 0.65 Aa	2.51 ± 0.64 Ab
60 days	3.26 ± 1.03 Aa	3.27 ± 0.68 Aa	2.58 ± 0.67 Ab
Full-mouth PI (% ± SD)	18.85 ± 14.76 Aa	20.18 ± 13.67 Aa	26.07 ± 12.66 Aa
Baseline	23.12 ± 15.88 Aa	26.66 ± 10.91 Aa	25.81 ± 14.19 Aa
60 days			
Full-mouth BOP (% ± SD)			
Baseline	17.15 ± 7.76 Aa	17.29 ± 7.41 Aa	17.68 ± 6.99 Aa
60 days	19.32 ± 10.40 Aa	18.51 ± 8.57 Aa	19.21 ± 6.61 Aa
Implant Region (n)			
Anterior	13	6	2
Posterior	12	33	26
p-value	0.002		
Implant Platform			
Tissue level	13	20	14
Bone Level	12	19	14
p-value	0.9890		
Insertion torque (n - %)			
> 35 N	10	9	6
15 N ≤ insertion torque ≤ 35 N	13	18	16
< 15 N	2	12	6
p-value	0.1903		
RFA (ISQ unit)			
At insertion (mean ± SD)	71.38 ± 5.28 Ba	72.00 ± 8.95 Ba	74.70 ± 6.95 Ba
At 60 days (mean ± SD)	77.31 ± 5.75 Aa	79.70 ± 4.45 Aa	80.20 ± 10.07 Aa

Distinct lowercase letters in a row indicate statistically significant difference by one-way ANOVA/Tukey's HSD test ($p < 0.05$). Gender, implant platform, implant region and insertion torque parameter frequencies were analyzed by χ^2 test.

Abbreviations: Probing depth (PD), relative clinical attachment level (rCAL), relative gingival margin position (rGMP), plaque index (PI), bleeding on probing (BOP), Resonance Frequency Analysis (RFA), Implant Stability Quotient (ISQ).

Table 2 – Intra- and intergroup comparisons of average (± SD) of peri-implant clinical parameters probing depth (PD), relative clinical attachment level (rCAL), and relative gingival margin position (rGMP).

Parameter	Aggressive Periodontitis	Chronic Periodontitis	Healthy
PD (mm - mean ± SD)			
30 days	2.65 ± 0.62 Aa	3.06 ± 0.78 Aa	2.69 ± 0.53 Aa
60 days	2.52 ± 0.60 Aa	2.53 ± 0.40 Aa	2.48 ± 0.35 Aa
rCAL (mm - mean ± SD)			
30 days	6.63 ± 1.57 Aa	6.14 ± 1.47 Aa	5.44 ± 1.96 Aa
60 days	6.42 ± 1.73 Aa	5.79 ± 1.39 Aa	5.30 ± 1.82 Aa
rGMP (mm - mean ± SD)			
30 days	3.94 ± 1.32 Aa	3.35 ± 1.15 Aa	2.82 ± 1.50 Aa
60 days	3.85 ± 1.57 Aa	3.26 ± 1.31 Aa	2.89 ± 1.65 Aa
% of implants presenting biofilm			
30 days	32%	30.76%	28.57%
60 days	24%	25.64%	25%
% of implants presenting BOP			
30 days	76%	69.23%	60.71%
60 days	64%	61.53%	64.29%

In comparisons of clinical implant distinct uppercase letters in a column and distinct lowercase letters in a row indicate statistically significant differences by ANOVA/Tukey HSD tests ($p \leq 0.05$). Frequency of implants presenting visible biofilm during the evaluations and frequency of implants presenting at least 1 site with bleeding on probing: no differences between the frequencies presented by each group were observed by $\chi 2$ ($p>0.05$).

Abbreviations: bleeding on probing (BOP).

Tabel 3. Univariate analysis for association between inflammatory cytokines and independent variables at 15 days.

Variables	**GM-CSF**	**IFN-y**	**IL-10**	**IL-12**	**IL-13**	**IL-1β**	**IL-4**	**IL-6**	**IL-8**	**TNF-α**
Gender	0,023	0,944	0,183	0,135	0,135	0,691	0,691	0,946	0,640	0,691
Age	0,658	0,754	0,753	0,432	0,864	0,460	0,593	0,669	0,669	0,112
Region	0,255	0,005	0,490	0,661	0,255	0,419	0,007	0,024	0,024	0,419
Type of platform	0,887	0,115	0,460	0,887	0,887	0,245	0,245	0,831	0,831	0,460
Lenght	0,974	0,501	0,197	0,974	0,184	0,610	0,702	0,542	0,542	0,629
Diameter of implant	0,481	0,527	0,158	0,555	0,489	0,612	0,108	0,023	0,023	0,444
Diameter of platform	0,609	0,039	0,964	0,086	0,062	0,340	0,230	0,041	0,041	0,510
Insertion Torque	0,240	0,011	0,110	0,048	0,736	0,460	0,096	0,298	0,298	0,538
Periodontal history	0,481	0,023	0,617	0,469	0,103	0,072	0,866	0,048	0,482	0,866
Ostell	0,537	0,659	0,719	0,865	0,967	0,868	0,748	0,725	0,725	0,223
PD	0,693	0,257	0,625	0,693	0,641	0,922	0,491	0,785	0,785	0,155
BOP	0,924	0,039	0,930	0,558	0,924	0,604	0,604	0,276	0,276	0,604
Biofilm	0,951	0,857	0,103	0,951	0,413	0,857	0,857	0,029	0,029	0,857

Abbreviatioins: Probing depth (PD), Bleeding on probing (BOP), GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN-y, Interferon gamma; IL-1β, Interleukin 1β; IL-4, Interleukin 4; IL-6, Interleukin 6; IL-8, Interleukin 8, Tumor necrosis factor alpha (TNF-α).

Table 4. Multiple logistic regression for cytokine release at 15 days.

Cytokine	Independent variable		Positive, %	OR	95% CI	*p* Value	pg/mL ± SD	p Value
GM-CSF	Gender	Female	95.24	Ref			0.163 ± 0.359	
		Male	4.76	0.107	0.012-0,967	0.047	0.03 ± 0.089	0.0339
IFN-y	Region	Anterior	10.87	Ref			0.258 ± 0.561	
		Posterior	89.13	5.143	1.481-17.86	0.01	1.639 ± 3.589	0.0035
	Torque	≤15 N	34.78	Ref			1.6 ± 1.71	
		15-35 N	43.48	0.106	0.024-0.466	0.003	1.431 ± 4.324	
		≥ 35 N	21.74	0.072	0.014-0.371	0.002	0.86 ± 1.657	0.0171
	BOP	≤ 24.90 %		Ref			0.99 ± 1.846	
		>24.90 %		4.357	1.497-12.686	0.007	1.494 ± 3.605	0.05
	Implant	Bone	56.52	Ref			1,91 ± 2.52	
		Tissue	43.48	0.279	0.099-0.791	0.016	0.805 ± 1.42	0.011
IL-1β	Periodontal History	Health	18	Ref			4.536 ± 9.626	
		Chronic	47.73	2.779	0.973-7.943	0.056	8.931 ± 13.423	
		Agressive	34.09	3.375	1.063-10.711	0.039	6.23 ± 11.49	0.0019
IL-4	Region	Anterior	11.36	Ref			0.673 ± 1.294	
		Posterior	88.64	4.303	1.414-13.101	0.01	2.241 ± 4.445	0.0032
IL-6	Periodontal History	Health	22.73	Ref			1.12 ± 2.47	
		Chronic	56.82	3.077	1.092-8.671	0.033	4.34 ± 2.73	
		Agressive	20.45	0.9	0.289-2.804	0.856	1.06 ± 2.53	0.0174
IL-8	Region	Anterior	13.64	Ref			234.64 ± 463.29	
		Posterior	86.36	3.149	1.063-9.335	0.039	995.69 ± 454.68	0.0073
	Biofilm	Absence	15.91	Ref			384.87 ± 444.38	
		Presence	84.09	2.904	1.027-8.212	0.044	2246.56 ± 747.69	0.0012

Abbreviations: BP, bleeding on probing; CI, confidence interval; GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN-y, Interferon gamma; IL-1β, Interleukin 1β; IL-4, Interleukin 4; IL-6, Interleukin 6; IL-8, Interleukin 8; OR, odds ratio; Ref, reference.

Tabela 5. Univariate analysis for association between inflammatory cytokines and independent variables at 60 days.

Variáveis	**GM-CSF**	**IFN-y**	**IL-10**	**IL-12**	**IL-13**	**IL1-β**	**IL-4**	**IL-6**	**IL-8**	**TNF-α**
Gender	0,477	0,350	0,350	0,673	0,942	0,350	0,161	0,206	0,161	0,350
Age	0,044	0,831	0,055	0,036	0,044	0,286	0,286	0,001	0,286	0,831
Region	0,661	0,080	0,211	0,922	0,661	0,024	0,080	0,258	0,080	0,803
Type of platform	0,451	0,669	0,669	0,920	0,451	0,669	1	0,528	0,200	0,393
Lenght	0,697	0,273	0,803	0,175	0,500	0,298	0,597	0,629	0,691	0,953
Implant diameter	0,220	0,417	0,567	0,441	0,070	0,189	0,693	0,926	0,567	0,567
Diameter of platform	0,668	0,010	0,578	0,985	0,628	0,906	0,026	0,569	0,369	0,662
Torque	0,109	0,060	0,659	0,009	0,541	0,912	0,160	0,525	0,056	0,457
Periodontal Condition	0,752	0,354	0,329	0,220	0,116	0,873	0,878	0,123	0,852	0,873
Ostell	0,360	0,395	0,549	0,324	0,704	0,867	0,278	0,267	0,882	0,680
PD	0,089	0,075	0,024	0,424	0,641	0,402	0,178	0,411	0,302	0,812
BOP	0,438	0,189	1	0,024	0,438	1	0,189	0,544	1	0,080
Biofilm	0,951	0,127	0,042	0,564	0,413	0,127	0,127	0,055	0,309	0,611

Abbreviatioins: Probing depth (PD), Bleeding on probing (BOP), GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN-y, Interferon gamma; IL-1β, Interleukin 1β; IL-4, Interleukin 4; IL-6, Interleukin 6; IL-8, Interleukin 8, Tumor necrosis factor alpha (TNF-α)

Table 6. Multiple logistic regression for cytokine release at 60 days.

Cytokine	Independent variable		Positive, %	OR	95% CI	*p* Value	pg/mL ± SD	p-value
GM-CSF	Age	≤ 44 years	71.43	Ref			0.291 ± 0.287	
		> 44 years	28.57	0.277	0.078-0.988	0.048	0.197 ± 0.368	0.0299
IFN-y	Platform	3.3 mm	2.39	Ref			1.27 ± 3.08	
		3.5 mm	9.52	8	0.598-10.69	0.116	0.62 ± 0.61	
		4.1 mm	30.95	9.42	1.023-12.85	0.015	1.75 ± 1.73	
		4.8 mm	5.38	19.5	1.777-21.39	0.048	2.07 ± 3.21	
		6.5 mm	4.76	1.5	0.109-2.675	0.762	1.16 ± 1.82	0.0061
IL-10	PD	≤ 2.5mm	65.91	Ref			6.27 ± 7.07	
		2.5-3mm	20.45	0.218	0.078-0.608	0.004	2.45 ± 2.88	
		>3mm	13.64	0.743	0.186-2.970	0.675	3.96 ± 4.19	0.0140
	Biofilm	Absence	13.64	Ref			5.39 ± 5.87	
		Presence	86.36	3.893	1.250-12.122	0.019	2.15 ± 2.78	0.001
IL-12	BOP	≤ 24.90 %	89.47	Ref			0.46 ± 0.39	
		>24.90 %	10.53	0.123	0.02-0.744	0.022	0.41 ± 0.32	0.406
	Age	≤ 44 years	73.68	Ref			0.53 ± 0.38	
		> 44 years	26.32	0.194	0.046-0.817	0.025	0.39 ± 0.65	0.0194
IL-13	Age	≤ 44 years	71.43	Ref			0.31 ± 0.35	
		> 44 years	28.57	0.277	0.078-0.988	0.048	0.24 ± 0.49	0.05
IL-1β	Region	Anterior	13.64	Ref			1.64 ± 2.69	
		Posterior	86.36	14.201	1.66-121.454	0.015	9.01 ± 3.75	0.0072
	Diameter	3.3 mm	25	Ref			4.42 ± 3.17	
		4.1 mm	45.45	0.097	0.012-0.813	0.031	3.69 ± 2.45	
		4.8 mm	29.55	0.198	0.02-1.968	0.167	3.22 ± 3.76	0.011
IL-6	Age	≤ 44 years	67.44	Ref			1.04 ± 2.27	
		> 44 years	32.56	0.197	0,077-0,503	0,001	0.35 ± 0.78	0.0011
	Biofilm	Absence	13.95	Ref			0.45 ± 0.52	
		Presence	86.05	3.376	1,056-10,792	0,04	0.62 ± 1.38	0.0462
IL-8	Torque	≤15 N	31.82	Ref			14638.21 ± 61409	
		15-35 N	40.91	0.247	0,076-0,809	0,021	9540.29 ± 6157.67	
		≥ 35 N	27.27	0.33	0,091-1,195	0,091	306.71 ± 360.79	

Abbreviations: BP, bleeding on probing; CI, confidence interval; GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN-y, Interferon gamma; IL-1β, Interleukin 1β; IL-6, Interleukin 6; IL-8, Interleukin 8; IL-10, Interleukin 10; IL-12, Interleukin 12; IL-13, Interleukin 13; OR, odds ratio; PD, probing depth; PI, plaque index; Ref, reference.

3 CONCLUSÃO

Diante dos resultados, pode-se concluir que diversas características relacionadas aos pacientes e aos implantes influenciam a liberação de marcadores inflamatórios no fluído crevicular peri-implantar durante a cicatrização inicial e osseointegração em pacientes com histórico de periodontite agressiva e crônica.

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Anexo 1 – Certificado de aprovação do Comitê de Ética em Pesquisa.

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Implantes dentais osseointegrados em pacientes com histórico de periodontite agressiva e crônica. Avaliação clínica, microbiológica e imunoenzimática"**, protocolo nº 017/2010, dos pesquisadores Renato Corrêa Viana Casarin, Hugo Felipe do Vale, Márcio Zaffallon Casati e Tiago Taiete, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 05/03/2010, com alterações em 30/10/2010.

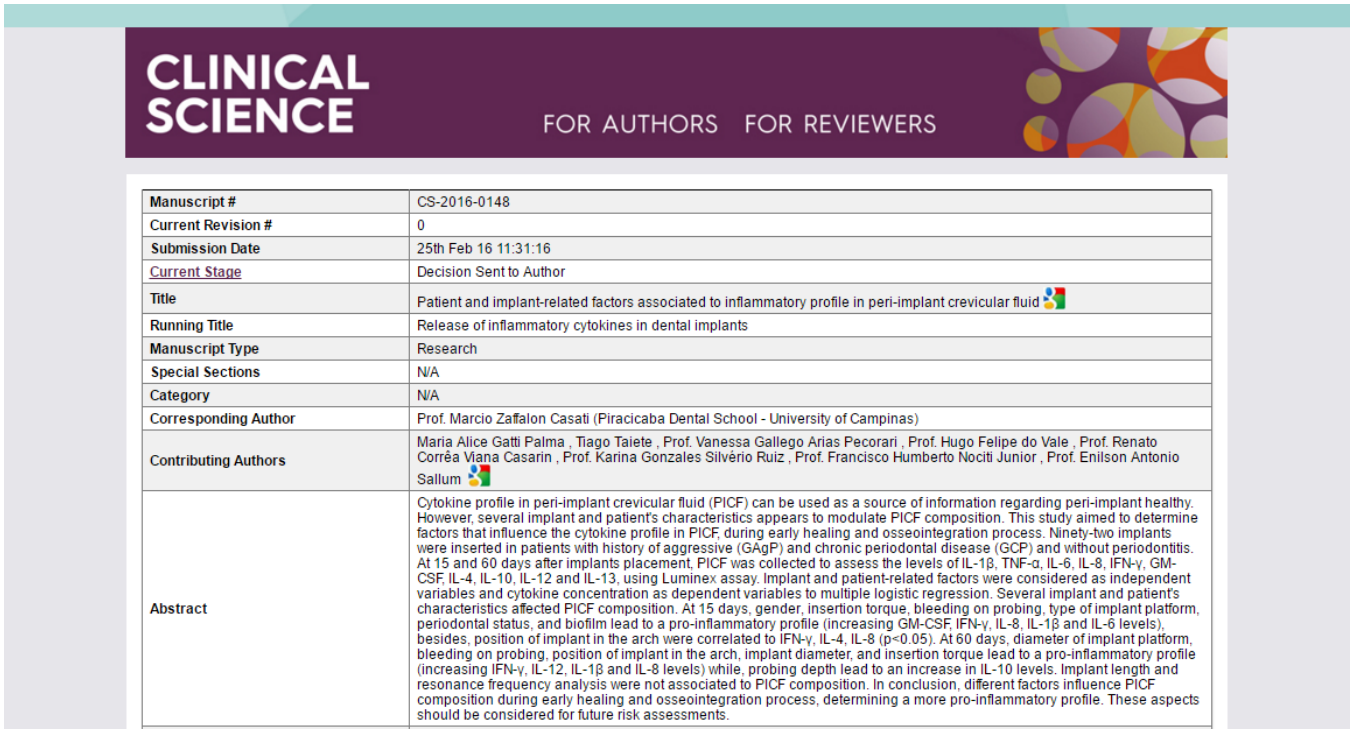
The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project **"Osseointegrated implants in patients with history of aggressive and chronic periodontitis. Clinical, microbiological and immunoenzymatic analysis"**, register number 017/2010, of Renato Corrêa Viana Casarin, Hugo Felipe do Vale, Márcio Zaffallon Casati and Tiago Taiete, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Mar 05, 2010; with alterations on Oct 30, 2010.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

Anexo 2- Comprovante de submissão do artigo na revista Clinical Science.



CLINICAL SCIENCE

FOR AUTHORS FOR REVIEWERS

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Abstract	Cytokine profile in peri-implant crevicular fluid (PICF) can be used as a source of information regarding peri-implant healthy. However, several implant and patient's characteristics appears to modulate PICF composition. This study aimed to determine factors that influence the cytokine profile in PICF, during early healing and osseointegration process. Ninety-two implants were inserted in patients with history of aggressive (GAgP) and chronic periodontal disease (GCP) and without periodontitis. At 15 and 60 days after implants placement, PICF was collected to assess the levels of IL-1β, TNF-α, IL-6, IL-8, IFN-γ, GM-CSF, IL-4, IL-10, IL-12 and IL-13, using Luminex assay. Implant and patient-related factors were considered as independent variables and cytokine concentration as dependent variables to multiple logistic regression. Several implant and patient's characteristics affected PICF composition. At 15 days, gender, insertion torque, bleeding on probing, type of implant platform, periodontal status, and biofilm lead to a pro-inflammatory profile (increasing GM-CSF, IFN-γ, IL-8, IL-1β and IL-6 levels), besides, position of implant in the arch were correlated to IFN-γ, IL-4, IL-8 ($p<0.05$). At 60 days, diameter of implant platform, bleeding on probing, position of implant in the arch, implant diameter, and insertion torque lead to a pro-inflammatory profile (increasing IFN-γ, IL-12, IL-1β and IL-8 levels) while, probing depth lead to an increase in IL-10 levels. Implant length and resonance frequency analysis were not associated to PICF composition. In conclusion, different factors influence PICF composition during early healing and osseointegration process, determining a more pro-inflammatory profile. These aspects should be considered for future risk assessments.