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Avaliação imunológica de duas sistemáticas de atenção periodontal na fase de manutenção periódica preventiva
- Análise secundária de uma subamostra de um ensaio clínico randomizado -

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“O período de maior ganho em conhecimento e experiência é o período mais difícil da vida de alguém.”

Dalai Lama

APRESENTAÇÃO

A presente investigação é parte integrante daquelas previstas em um projeto maior, aqui denominado *Projeto Principal* (*O efeito do controle do biofilme supragengival e da combinação do controle do biofilme supra e subgengival na saúde periodontal de pacientes participantes de um programa de manutenção periodontal preventiva - Um ensaio clínico randomizado)*.

O *Projeto Principal* foi aprovado pela Comissão de Pesquisa da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul (UFRGS) e Comitê Central de Ética em Pesquisa da UFRGS (Anexos 1 e 2).

RESUMO

STADLER, Amanda Finger. **Avaliação imunológica de duas sistemáticas de atenção periodontal na fase de manutenção periódica preventiva - análise secundária de uma subamostra de um ensaio clínico randomizado**. 2015. 122 f. Tese de Doutorado – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2015.

Introdução: A necessidade de pacientes serem inseridos em protocolos de manutenção periodontais (PMP) após tratamento periodontal é bem estabelecida na literatura. No entanto, ainda não é bem estabelecido quais procedimentos são necessários para manter a saúde periodontal. O objetivo do presente estudo foi identificar quais os biomarcadores relacionados com a doença periodontal e comparar o efeito de dois PMPs nos níveis destes biomarcadores pró e anti-inflamatórios do fluido crevicular gengival (FCG) durante 1 ano. Materiais e Métodos: Após o tratamento periodontal não-cirúrgico, 34 sujeitos (14H/20M, idade média 54 anos) diagnosticados com periodontite crônica foram randomicamente alocados em um dos seguintes grupos: a) somente raspagem supragingival; b) raspagem supra e subgengival. Todos os participantes receberam instruções de higiene oral personalizados e polimento dos dentes. Os indivíduos foram vistos em intervalos de três meses para a realização de exames clínicos, coleta de FCG e procedimentos de manutenção periodontal. As principais citocinas pró- e anti-inflamatórias e quimiocina relacionadas com a doença periodontal foram identificadas por meio de uma revisão sistemática da literatura com metanálise. Em seguida, os níveis de FCG de 16 citocinas e quimiocinas dos 34 sujeitos incluídos no estudo foram medidos por meio de um imunoensaio múltiplo. Resultados: Não foram observadas diferenças significativas nos parâmetros clínicos ou imunológicos entre os dois PMPs. Os níveis médios de concentração no FCG da maioria das citocinas pró-inflamatórias foram geralmente baixos / moderados durante todo o período de estudo, com exceção da IL-1β. Os níveis das citocinas anti-inflamatórias IL-4 e IL-13 foram moderado / alto. Entre as quimiocinas, os níveis de MIP-1α e MCP-1 foram altos, e a concentração de IL-8 foi muito alta durante todo o estudo. Conclusões: Os resultados sugerem que um PMP baseado apenas no controle do biofilme supragengival é tão eficaz na manutenção de baixos níveis de inflamação periodontal e estabilidade clínica após o tratamento quanto um PMP baseado em controle supra e subgengival.

Palavras-chave: periodontite; doenças periodontais; raspagem dentária; manutenção preventiva; citocinas; quimiocinas; imunologia.

ABSTRACT

STADLER, Amanda Finger. **Immunological evaluation of two protocols of periodontal care during preventive periodic maintenance phase – a subsample secondary analysis of a randomized clinical trial.** 2015. 122 f. PhD Thesis – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2015.

Introduction: The need for patients to be enrolled into periodontal maintenance protocols (PMP) after periodontal treatment is well established in the literature. However, it is still unknown which interventions are necessary to maintain periodontal health. The aim of the present study was to identify the biomarkers related to periodontal disease, and to compare the 1-year effect of two periodontal maintenance protocols (PMP) on gingival crevicular fluid (GCF) levels of pro- and anti-inflammatory biomarkers. Material and Methods: Following non-surgical periodontal treatment, 34 subjects (14M/20F, mean age: 54 years) diagnosed with chronic periodontitis were randomly assigned into one of the following study arms: a) supragingival scaling only; b) supra and subgingival scaling. All subjects received personalized oral hygiene instructions and tooth polishing. Subjects were seen at 3 months intervals for periodontal maintenance, clinical data and GCF collection. The major pro-and anti-inflammatory cytokines and chemokine related to periodontal disease were identified through a systematic review of the literature with meta-analysis. After that, GCF levels of 16 different cytokines and chemokines from the 34 subjects included in the study were measured using a multiplex immunoassay. Results: No significant differences between PMPs were observed for any clinical parameters or immunological biomarkers. Median GCF concentration levels for most pro-inflammatory cytokines were generally low/moderate throughout the study period, with the exception of IL-1β. GCF levels of anti-inflammatory cytokines IL-4 and IL-13 were moderate/high. For chemokines, GCF levels for MIP-1α and MCP-1 were high, and concentration for IL-8 was very high. Conclusion: Our findings suggest that a PMP based on supragingival biofilm control alone is as effective in maintaining low levels of periodontal inflammation and clinical stability after treatment as a PMP based on combined supra/subgingival biofilm control.

Keywords: periodontitis; periodontal diseases; dental scaling; preventive maintenance; cytokines; chemokines; immunology.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

BOP	bleeding on probing
CAL	clinical attachment loss
ChP	chronic periodontitis
DP	doença periodontal
ELISA	enzyme-linked immunosorbent assay
FCG	fluido crevicular gengival
GCF	gingival crevicular fluid
GM-CSF	granulocyte macrophage colony-stimulating factor (fator estimulador de colônias de macrófagos)
IFN	interferon
IL	interleucina/interleukin
MCP/CCL2	monocyte chemotactic protein (proteina quimiotática de monócitos)
MMP	matrix metalloproteinase (metaloproteinase de matriz)
MPP	manutenção periódica preventiva
OPG	osteoprotegerina
PCR	reação em cadeia da polimerase
PD	periodontal disease
PI	perda de inserção
PMN	células polimorfonucleares
PMP	periodontal maintenance protocols (protocolos de manutenção periodontal)
PPD	periodontal pocket depht
PS	profundidade de sondagem
RANK	receptor activator of nuclear factor kappa-B (receptor ativador do fator nuclear kappa Beta)
RANKL	receptor activator of nuclear factor kappa-B ligand (ligante do receptor ativador do fator nuclear kappa Beta)
SS	sangramento à sondagem
T_0	linfócitos T virgem
T_h	linfócitos T auxiliaries (helper)
Treg	linfócitos T regulatórios (regulatory)
TGF	transforming growth factor (fator de transformação de crescimento)

TIMP	tissue inhibitor of metalloproteinases (inibidor tecidual de metaloproteinases)
TNF	tumor necrosis factor (fator de necrose tumoral)

SUMÁRIO

1 INTRODUÇÃO AO TEMA

1.1 INTRODUÇÃO

A doença periodontal é um processo patológico que afeta o periodonto, caracterizada pela inflamação da gengiva (gengivite) e destruição dos tecidos de suporte dos dentes (periodontite)[1]. Quando consideradas as formas leve e moderada, a prevalência da gengivite se aproxima de 100%. Já a prevalência da periodontite na América do Sul varia de 4% a 19%[2]. Dados recentes mostram que a forma grave da periodontite é considerada a sexta doença mais prevalente no mundo, afetando 10,8% da população, estando o Brasil entre os países com prevalência maior do que a média global[3].

A principal causa da doença periodontal é a presença de bactérias periodontopatogênicas, principalmente Gram-negativas, organizadas em um biofilme[4]. A partir do estabelecimento deste biofilme, sua maturação e manutenção sem ser desorganizado mecanicamente, haverá o desenvolvimento da gengivite. Este quadro inflamatório leva ao aumento da profundidade de sondagem, permitindo que bactérias anaeróbias e mais patogênicas tornem-se predominantes. As condições supragengivais, como a presença de depósitos bacterianos e gengivite, podem modular o estabelecimento e desenvolvimento do biofilme subgengival[5-8]. Desta forma, as bactérias e seus metabólitos estimulam uma resposta inflamatória do hospedeiro, e a exacerbação desta resposta pode causar degradação de tecido conjuntivo e reabsorção óssea, podendo levar à perda dentária (Figura 1)[9, 10].

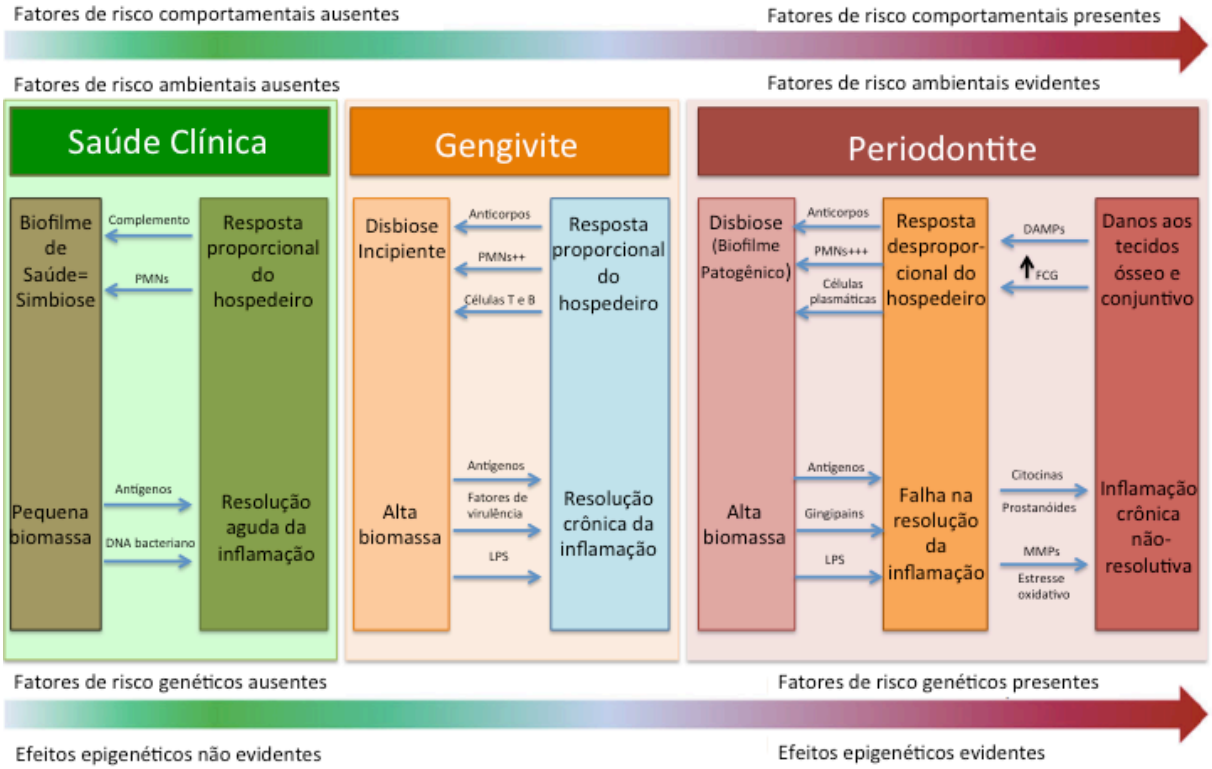
O tratamento da doença periodontal é fundamentalmente mecânico e não-cirúrgico, buscando-se controlar e restringir a presença do biofilme bacteriano sobre a superfície dentária tanto supragengival quanto subgengival[11, 12]. No entanto, os resultados terapêuticos podem falhar especialmente na ausência de um controle adequado do biofilme supragengival[13], o qual pode levar a um novo aumento dos níveis de inflamação local. Desta forma, sugere-se que os pacientes periodontais tratados sejam envolvidos em uma fase conhecida como Manutenção Periódica Preventiva (MPP) ou Terapia Periodontal de Suporte para que o monitoramento dos resultados obtidos seja constante[13-15].

A literatura tem mostrado que pacientes que mantém um bom controle do biofilme associado a consultas regulares de manutenção mantém índices clínicos periodontais compatíveis com saúde e, consequentemente, perdem menos dentes[16].

Nas consultas de MPP são realizadas intervenções a critério do profissional, podendo ser realizadas intervenções voltadas para a área subgengival ou buscando o efetivo controle diário do biofilme supragengival[17]. A relativa importância de cada uma destas intervenções ainda não está bem clara. A literatura apresenta um ensaio clínico que compara diretamente estes dois procedimentos, mostrando que não houve diferença nos parâmetros clínicos da doença entre os dois grupos por um período de 1 ano[18]. No entanto, o índice de placa dos pacientes incluídos foi medido apenas ao final do estudo e mostrou-se elevado (0.58±0.1 no grupo que recebeu raspagem subgengival, e 0,53±0.13 no grupo que recebeu apenas raspagem supragengival). Além disso, parâmetros microbiológicos e imunológicos não foram analisados na amostra.

Desta forma, o objetivo desta tese foi comparar o efeito de duas estratégias de intervenção periodontal profissional (procedimentos realizados pelo binômio paciente-profissional buscando o controle supragengival isoladamente versus os mesmos procedimentos de controle supra acrescidos do controle profissional do biofilme subgengival) em pacientes periodontais tratados Uma vez que a resposta imunológica é a responsável pela destruição periodontal, e que os sinais inflamatórios aparecem precocemente à destruição tecidual[10, 19], foram identificadas as principais citocinas pró- e anti-inflamatórias e quimiocinas que caracterizam a doença periodontal, e então foram comparados estes biomarcadores imunológicos presentes no fluido crevicular gengival nos dois grupos.

Figura 1 - Modelo da doença periodontal.



A presença de bactérias estimula uma resposta imuno-inflamatória do hospedeiro, a qual estimula uma cascata de reações inflamatórias que leva a destruição dos tecidos de suporte dos dentes (adaptado de Meyle e Chapple, 2015). PMNs: neutrófilos polimorfonucleares; LPS: lipopolissacarídeos; FCG: fluido crevicular gengival; DAMPs: padrões moleculares associados a danos; MMPs: metaloproteinases de matriz.

1.2 REVISÃO DA LITERATURA

1.2.1 O tratamento periodontal

O objetivo do tratamento periodontal é a cura da doença, por meio da desorganização do biofilme e eliminação total ou parcial da carga bacteriana nos sítios doentes, diminuindo, assim, a resposta inflamatória do hospedeiro, assim como a eliminação da presença de fatores retentivos de placa, como por exemplo o cálculo dentário. Desta forma, o tratamento da doença periodontal é realizado de forma a incluir o controle mecânico não-cirúrgico do biofilme supragengival e subgengival, seguido do alisamento e polimento das superfícies dentárias[11, 12].

A eficácia do controle do biofilme subgengival para o tratamento periodontal vem sido demonstrada ao longo do tempo. Kaldahl et al. (1996)[20] compararam os efeitos de 4 modalidades de tratamento periodontal (raspagem supragengival, raspagem supra e subgengival, retalho de Widman modificado e retalho com ressecção óssea) em um estudo do tipo boca dividida em 82 pacientes. Os resultados mostraram que as 4 modalidades diminuíram a profundidade de bolsas periodontal e aumentaram a inserção clínica, no entanto a raspagem supra e subgengival foi mais efetiva do que a raspagem subgengival apenas. Também em um estudo do tipo boca dividida, Westfelt et al. (1998)[5] compararam o efeito do controle do biofilme supragengival realizado sozinho e em conjunto com o controle subgengival. Os resultados mostraram que a associação dos controles supra e subgengival apresentou maior diminuição de profundidade de sondagem e aumento de inserção clínica, demonstrando a importância do controle subgengival para um tratamento efetivo. Van der Weidjen e Timmerman (2002)[21] realizaram uma revisão sistemática com o objetivo de avaliar os efeitos da intervenção subgengival nos parâmetros clínicos de pacientes com periodontite crônica. A análise de 26 artigos mostrou que a média ponderada de ganho de inserção clínica em bolsas com profundidade de sondagem inicial ≥ 5mm foi de 0,64mm para os pacientes que receberam raspagem subgengival associada a raspagem supragengival, comparado a 0,37mm para os pacientes que receberam apenas controle de placa supragengival. Os resultados deste estudo permitiram concluir que, em pacientes com periodontite crônica, o controle do biofilme subgengival em conjunto com o supragengival é um tratamento efetivo em reduzir profundidade de sondagem e

ganhar inserção clínica, sendo mais efetivo do que o controle do biofilme supragengival per se.

A importância do controle do biofilme supragengival no tratamento periodontal tem sido evidenciada nas últimas décadas. Hellstrom et al. (1996)[22] observaram efeitos significantes da intervenção profissional supragengival frequente combinada com um adequado controle do biofilme pelo paciente na microbiota subgengival de bolsas periodontais moderadas a profundas. Uma série de estudos realizada por Gomes e colaboradores também vem demonstrando a importância do controle do biofilme supragengival. No estudo de Gomes et al. (2007)[23], 50 pacientes foram tratados apenas com intervenção profissional supragengival e instrução de higiene oral. Após 180 dias de acompanhamento, as médias de profundidade de sondagem em sítios profundos e moderados diminuíram de 6,8 e 4,6 mm para 4,4 e 2,9 mm para não-fumantes e de 6,6 e 4,6 mm para 4,5 e 3,1 mm para pacientes fumantes. Os resultados permitiram concluir que o controle de placa supragengival pode melhorar substancialmente a saúde periodontal e reduzir as necessidades de tratamentos mais caros e complexos. Este mecanismo pôde ser explicado no estudo subsequente de Gomes et al. (2008)[24], quando os autores avaliaram o efeito do controle do biofilme supragengival na microbiota subgengival dos mesmos pacientes. Coletas de biofilme subgengival foram realizadas ao início e 30, 90 e 180 dias após a raspagem supragengival, com o auxílio de pontas de papel absorvente. As amostras foram avaliadas por meio da técnica de reação em cadeia da polimerase em tempo real (Real Time PCR*)* e os resultados mostraram que houve uma redução significativa na contagem de microbiota subgengival. Posteriormente, em um estudo do tipo boca dividida, Gomes et al. (2014)[25] mostraram que o controle profissional do biofilme supragengival realizado em uma fase separada e anterior ao controle subgengival diminui a necessidade de intervenção subgengival em 48%.

No entanto, a literatura tem mostrado que a terapia periodontal pode falhar, especialmente na ausência de um bom controle de placa pelo paciente e na frequência diminuída de consultas de manutenção[13]. Ainda na década de 70, Nyman et al. (1975)[26] reportaram que um grupo de pacientes que recebiam orientação de higiene oral e controle profissional do biofilme supragengival frequentes (a cada 15 dias) apresentou redução na profundidade de sondagem, sem evidências de perda de inserção clínica ao final de 2 anos. Já um grupo de pacientes que recebeu apenas controle profissional do biofilme supragengival com menor frequência (a

cada 6 meses) apresentou recidiva de bolsas periodontais e perda de inserção clínica ao final dos 2 anos de estudo. Posteriormente, Rosling et al. (1976)[27] mostraram que pacientes tratados que receberam intervenção profissional a cada 2 meses mostraram evidência de reparo de defeitos infra-ósseos, já pacientes que receberam intervenção anual apresentaram recidiva de doença, com perda progressiva de inserção periodontal. Lindhe e Nyman (1984)[16] realizaram um estudo longitudinal de 14 anos de duração, mostrando que pacientes que mantiveram um bom controle de placa (demonstrado pelos baixos valores nos Índices Gengival e de Placa durante o curso do estudo) apresentaram baixo índice de recidiva da doença periodontal.

Neste sentido, observa-se a importância do acompanhamento longitudinal do indivíduo tratado para que os eventuais desequilíbrios no eixo etiopatogênico possam, de imediato, ser diagnosticados, buscando manter os resultados após terapia ativa.

1.2.2 A Manutenção Periódica Preventiva

Segundo o glossário da Sociedade Brasileira de Periodontologia, a manutenção do tratamento é definida como Terapia Periodontal de Suporte ou Manutenção Periódica, a qual engloba “procedimentos realizados em intervalos selecionados para ajudar o indivíduo a manter sua saúde bucal”[28]. No entanto, o termo Manutenção Periódica Preventiva (MPP), sugerido pela Academia Americana de Periodontia [1], é o adotado neste estudo.

Em 1987, Ramfjord[29] observou, por meio de uma revisão da literatura associada a achados clínicos, que diferentes técnicas cirúrgicas de tratamento apresentavam resultados semelhantes e independentes dos padrões de controle do biofilme pelos pacientes, desde que estes participassem de uma sessão de controle profissional dos biofilmes supra e subgengival a cada 3 meses. Esta periodicidade é, atualmente, recomendada pela Academia Americana de Periodontia, já que este período é aceito como suficiente para reduzir a probabilidade de ocorrer progressão da doença. No entanto, esta periodicidade deve ser individualizada, suprindo as necessidades de cada paciente[17].

A importância da MPP foi claramente demonstrada no estudo longitudinal de Axelsson et al. (2004)[30], o qual avaliou 257 pacientes após 30 anos de tratamento dental preventivo. Os pacientes foram acompanhados bimensalmente pelos

primeiros 2 anos e trimestralmente durante os 4 anos seguintes, e receberam orientação de higiene bucal individualizada e limpeza mecânica profissional, realizada por higienistas. Neste ponto os pacientes foram separados em 3 grupos de acordo com o risco de progressão de doença. No período subsequente, o grupo de risco baixo foi acompanhado anualmente, o grupo de risco intermediário foi acompanhado a cada 6 meses e o grupo de alto risco foi acompanhado a cada 3 meses. Os resultados mostraram que a incidência de cárie, doença periodontal e perda dentária foi muito baixa nestes pacientes, sendo que a principal causa de perda dentária não foi progressão da doença, mas sim fratura radicular, para todos os grupos. Além disso, muitos sítios não exibiram perda de inserção clínica ao longo dos 30 anos. Lorentz et al, 2009[31], em um estudo prospectivo com 150 sujeitos, também demonstram que um programa de manutenção periodontal pode estabilizar a condição periodontal obtida após o tratamento periodontal, assim como controlar a ação de fatores de risco para a progressão da doença. Os pacientes foram tratados e acompanhados a cada 3 meses, por um período de 12 meses. Nas consultas de manutenção, os pacientes receberam instrução de higiene bucal e controle profissional do biofilme supragengival. Ao final do estudo, 86,7% dos pacientes apresentaram uma condição periodontal estável.

Neste sentido, a literatura tem demonstrado que pacientes sob MPP apresentam menor taxa de perda dentária quando comparados a pacientes que não realizam ou não apresentam uma periodicidade de consultas de manutenção[32, 33]. Já na década de 70, o estudo de Hirschfeld e Wasserman (1978)[34], no qual os autores analisaram perdas dentárias em 600 pacientes tratados em clínica privada e acompanhados por um período que variou entre 15 e 39 anos, demonstrou que a maior incidência de perda dentária ocorre entre aqueles pacientes que não participam de um programa de manutenção adequado. Mais atualmente, a revisão sistemática de Chambrone *et al*., (2010)[35] levantou dados de 13 estudos, que avaliaram um total de 41404 dentes de pacientes tratados e submetidos a manutenção. Observou-se que aproximadamente 4000 dentes foram perdidos, sendo a taxa de perda relacionada à progressão de doença periodontal variando de 1,5% e 9,8%. Por outro lado, o percentual de pacientes participantes de programa de MPP que não experimentaram qualquer perda dental variou entre 36% e 88,5%. Pode-se observar que, quando se dimensiona o resultado da manutenção periodontal por um desfecho real, no caso de perdas dentárias, torna-se evidente

que pacientes que participam de programas de manutenção periodontal perdem menos dentes.

Em relação aos procedimentos que devem ser realizados durante a fase de manutenção periódica preventiva, a Academia Americana de Periodontia apresenta um guia de procedimentos, no qual chama a atenção para a necessidade da remoção de placa e cálculo dentário, além de modificações comportamentais como orientação de higiene bucal e aconselhamento sobre os fatores de risco da doença, como por exemplo o fumo. Além disso, este guia também sugere a realização de raspagem e alisamento radicular, se indicado[36]. No entanto, surge o questionamento de quando esta raspagem subgengival é indicada e necessária.

Com o intuito de avaliar a efetividade de duas abordagens de MPP (uma com controle apenas supra e outro com controle supra e subgengival do biofilme), Heasman et al. (2002)[37], realizaram uma revisão sistemática da literatura. A busca por artigos localizou apenas um ensaio clínico comparando diretamente a realização do controle profissional do biofilme supragengival com o controle profissional do biofilme subgengival durante o período de MPP. Esta evidência citada na revisão sistemática foi o estudo de Jenkins et al. (2000)[18], o qual avaliou, por meio de um estudo prospectivo, o efeito do controle subgengival em 31 pacientes sob um programa de MPP durante 12 meses. Os pacientes foram previamente tratados e então divididos em 2 grupos para a fase de MPP: um grupo recebeu apenas controle supragengival e outro recebeu, além do controle supragengival, controle subgengival do biofilme, realizado por profissionais, com utilização de instrumentos manuais, a cada 3 meses durante 12 meses. Ambos os grupos receberam instrução de higiene oral em todas as consultas. Os resultados não mostraram alterações significativas de profundidade de sondagem, nível relativo de inserção e sangramento a sondagem entre os dois grupos em nenhum tempo experimental. Da mesma foram, também não houve alteração significativa dos valores de parâmetros clínicos ao longo do tempo. O Índice de Placa dos sujeitos foi medido apenas ao final do estudo, e mostrou que não houve diferença significativa entre os grupos. Estes resultados colocam em dúvida a necessidade de seguidas intervenções subgengivais em pacientes aderentes a um programa de MPP.

No entanto, os efeitos de ambas as modalidades de controle pode se dar não somente nos parâmetros clínicos, mas também nos parâmetros microbiológicos e imunológicos. O estudo citado valeu-se apenas de parâmetros clínicos, não

relatando se existe alteração na microbiota subgengival ou nos biomarcadores inflamatórios destes pacientes. Desta forma, esta tese se propõe a fazer uma análise imunológica dos resultados de dois protocolos de manutenção periodontal semelhantes ao estudo supracitado, uma vez que a literatura mostra que é a partir da resposta inflamatória que se dá a destruição periodontal[10].

1.2.3 Parâmetros imunológicos da doença periodontal

1.2.3.1 A resposta inflamatória na doença periodontal

Durante o primeiro estágio da resposta inflamatória não existem sinais clínicos evidentes de inflamação[38, 39]. As bactérias e seus metabólitos são reconhecidos por células residentes do hospedeiro, sendo as células epiteliais as primeiras a reconhecer esta invasão, por meio de receptores celulares de superfície[40]. Tão logo as células epiteliais reconhecem um invasor, elas estimulam a produção de interleucina (IL) -8, uma importante quimiocina que estimula o recrutamento de neutrófilos[41]. As bactérias que conseguem ultrapassar a primeira barreira celular de defesa são reconhecidas por macrófagos, os quais estimulam citocinas (principalmente IL-1, IL-6 e fator de necrose tumoral alpha – TNF-α) a atraírem outras células polimorfonucleares (PMN) de defesa[38, 42]. Ao mesmo tempo, células apresentadoras de antígenos (principalmente células dendríticas) também reconhecem bactérias que porventura invadiram o epitélio da bolsa, estimulando a liberação de citocinas que ativam células específicas de defesa, tais como linfócitos B e linfócitos T.

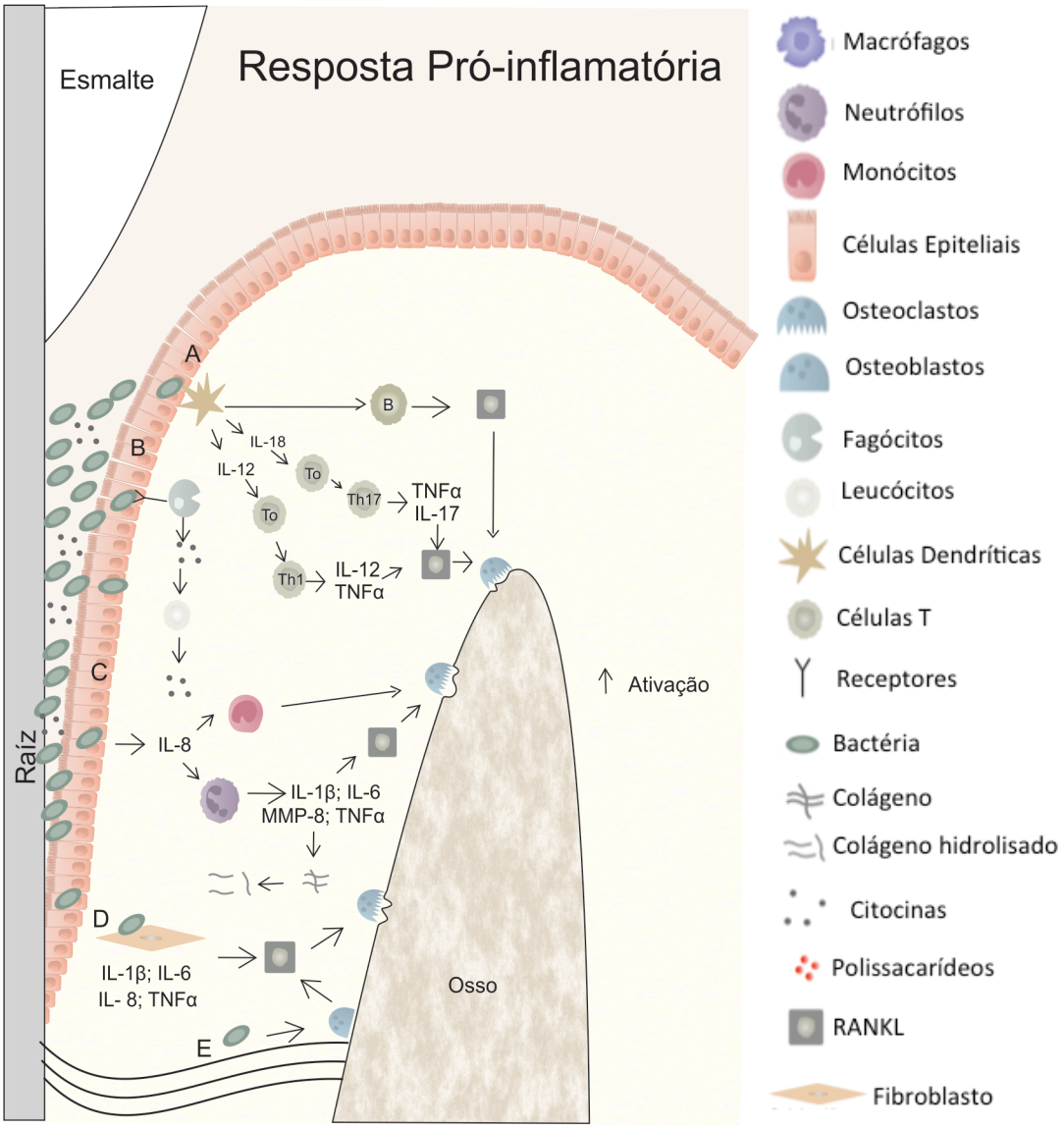
Citocinas pró-inflamatórias estimulam os PMNs e outras células a produzirem metaloproteinases de matriz (MMP) que são capazes de degradar a matriz extracelular. Por exemplo, MMP-2, -8 e -9 são produzidas, respectivamente, por fibroblastos, neutrófilos e PMNs e são capazes de degradar colágeno[43-45]. Outra importante citocina, TNF-α, é capaz de ativar a secreção do ligante do receptor do ativador do fator nuclear kappa B (RANK), conhecido como RANKL. Esta citocina se liga ao receptor RANK dos pré-osteoclastos, ativando e induzindo a maturação destas células, resultando em osteoclastogênese e consequentemente reabsorção óssea[42, 46, 47] (Figura 2).

Ao mesmo tempo em que este processo pró-inflamatório ocorre, o hospedeiro tenta promover homeostase por meio de uma resposta anti-inflamatória (Figura 3).

Citocinas anti-inflamatórias como IL-4 e IL-10 bloqueiam a produção de citocinas pró-inflamatórias pelos linfócitos T_{h1} e induzem a secreção de inibidor tecidual de metaloproteinases (TIMP) e de osteoprotegerina (OPG – citocina que inibe a ligação entre RANK e RANKL), inibindo assim a osteoclastogênese. Estas citocinas também são capazes de agir por retroalimentação, aumentando a estimulação de células T_{h2}[48]. Outras citocinas, como a IL-13, estimulam a produção de colágeno pelos fibroblastos[42] e também estimulam a biossíntese de lipoxinas pelos ácidos araquidônicos[49].

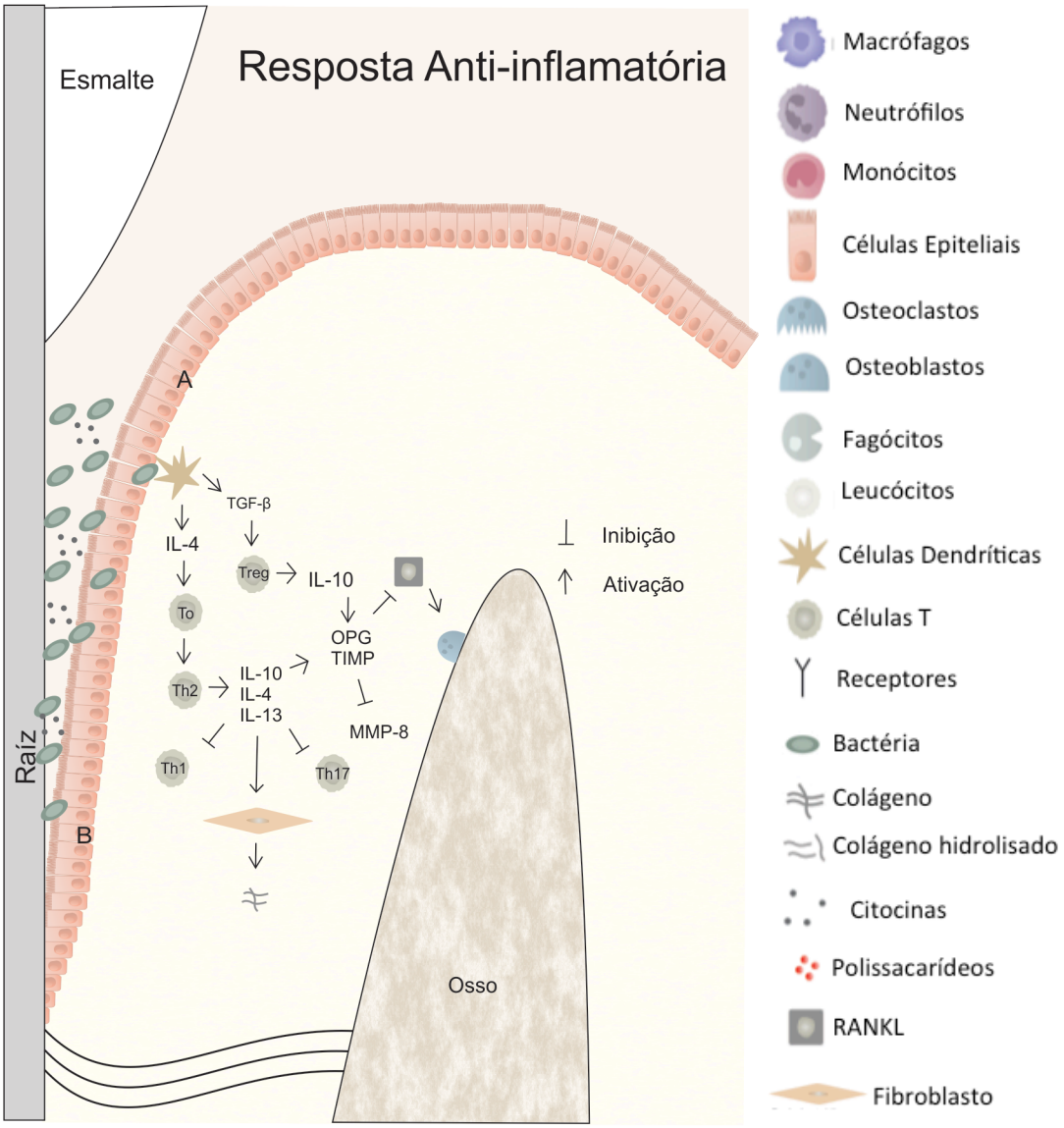
Recentemente, o papel de alguns agonistas que ativamente promovem resolução do processo inflamatório tem sido estudado[49-51]. Estes mediadores agem de forma diferente dos mediadores anti-inflamatórios previamente descritos, os quais são antagonistas que limitam a duração e magnitude da resposta inflamatória. A cascata de pró-resolução é iniciada após uma resposta inflamatória aguda que origina mediadores lipídicos, principalmente lipoxinas[52, 53]. Lipoxinas são biossintetizadas a partir do ácido araquidônico pelas células epiteliais, monócitos e eosinófilos quando expostos a citocinas anti-inflamatórias, tais como IL-4 e IL-13. As lipoxinas também podem inibir a secreção de citocinas e quimiocinas pró-inflamatórias pelas células T_h, tais como a IL-1β, TNF-α e IL-8, ao mesmo tempo em que estimulam a produção de IL-4[54].

Figura 2. Resposta pró-inflamatória local na doença periodontal



A: Células dendríticas reconhecem a presença de bactérias e seus substratos, estimulando células de defesa como os linfócitos B e também a liberação de IL-12 e IL-18, que estimulam o amadurecimento dos linfócitos T_0 em T_{h1} e T_{h17} respectivamente. Estas citocinas ativam a citocina RANKL, a qual estimula a atividade dos osteoclastos. B: macrófagos e fagócitos reconhecem a invasão bacteriana por meio de seus receptores de superfície e produzem citocinas e quimiocinas que atraem células PMNs, as quais estimulam ainda mais a secreção de citocinas pró-inflamatórias. C: células epiteliais reconhecem a presença bacteriana e estimulam a produção da quimiocina IL-8, que ativam neutrófilos e monócitos, os quais estimulam a atividade da RANKL. D: Fibrobalastos também são capazes de reconhecer bactérias invasoras, estimulando a secreção de citocinas pró-inflamatórias, as quais ativam a RANKL. E: osteoblastos são capazes de reconhecer bactérias e ativar a atividade de RANKL. (AFS, 2015)

Figura 3. Resposta anti-inflamatória local na doença periodontal.



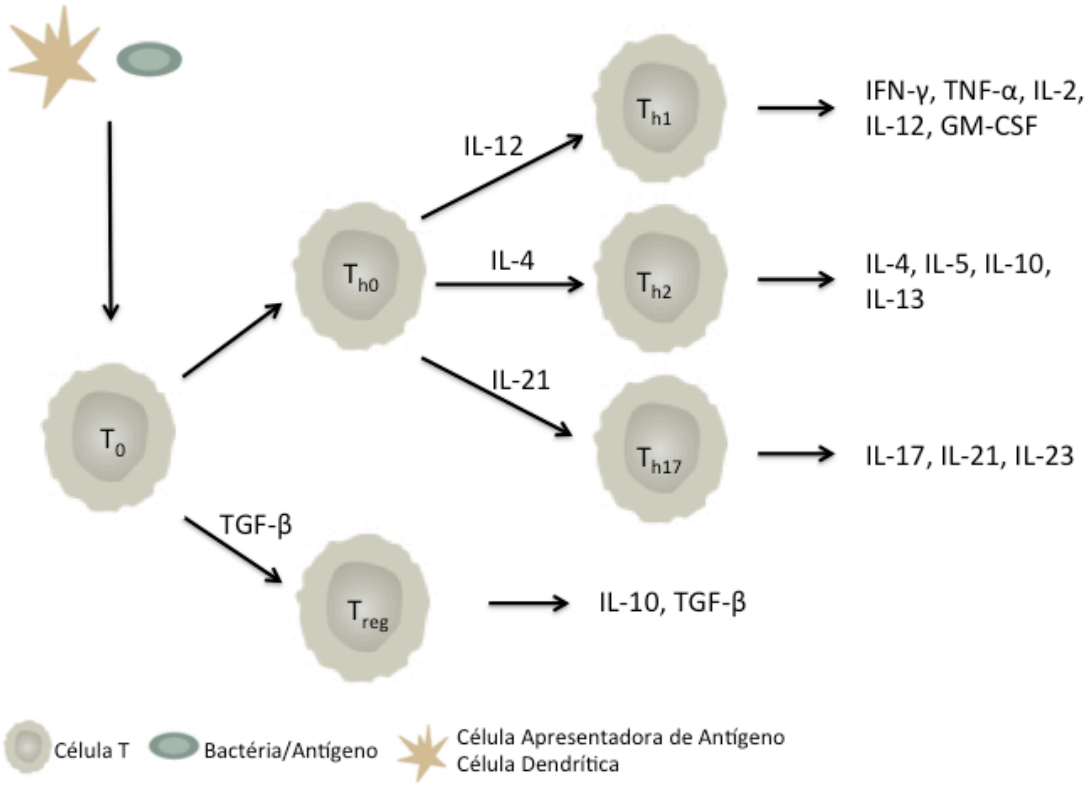
A: Células dendríticas reconhecem bactérias invasoras e estimulam a secreção de citocinas anti-inflamatórias IL-4 e TGF-b, que estimulam o amadurecimento dos linfócitos T_0 em linfócitos T_{h2} e T_{reg} respectivamente. Estes linfócitos secretam IL-10 que induz a secreção de TIMP e de OPG, inibindo a osteoclastogênese. B: Os Linfócitos T_{H2} também estimulam a secreção de IL-4, que bloqueiam a produção de citocinas pró-inflamatórias pelos linfócitos T_{h1} e $T_{H17,}$ e de IL-13, que estimula a síntese de colágeno pelos fibroblastos. (AFS, 2015)

1.2.3.2 O papel das citocinas na resposta inflamatória

Após o estabelecimento de um biofilme no sulco gengival, a resposta inflamatória do hospedeiro à invasão microbiana é mediada principalmente por citocinas[38, 41, 55, 56]. Citocinas são proteínas solúveis de baixo peso molecular produzidas por vários tipos de células que agem como mediadores de homeostase e imunidade, tendo como principal função a comunicação celular[57]. A nomenclatura das citocinas pode ser dada a partir de sua atividade biológica, como é o caso das quimiocinas (citocinas quimioatrativas, responsáveis pelo recrutamento de leucócitos para os sítios infectados) e do Fator de Necrose Tumoral (TNF), ou a partir da célula de origem, como por exemplo as interleucinas e linfocinas (citocinas produzidas principalmente por leucócitos e linfócitos, respectivamente)[58].

O genoma humano possui mais de 180 genes capazes de codificar citocinas[58]. As principais células secretoras de citocinas são os linfócitos T. Tão logo uma célula apresentadora de antígeno (principalmente células dendríticas) reconhecem um agente agressor, elas ativam os fatores de transcrição das células T virgem (T_0). Estes fatores de transcrição agem no núcleo celular, promovendo a ativação do gene codificador de citocinas via RNA mensageiro[58]. Desta forma, a célula T_0 secreta citocinas que poderão agir num sistema de retroalimentação, estimulando o amadurecimento das células T_0 em células T auxiliares (T_{h0}) ou regulatórias (T_{reg}). Dependendo do tipo de citocinas produzidas, a maturação dos linfócitos T será diferenciada para um subtipo de célula T_{h0}[59], entre eles os linfócitos T_{h1}, T_{h2}, $T_{h17,}$ ou para o subtipo T_{reg}. Cada subgrupo diferente de linfócito estimula a secreção de grupos diferentes de citocinas (Figura 4)[60]. Quando os linfócitos T_{h0} são expostos à IL-12, eles são diferenciados em linfócitos T_{h1}, os quais secretam citocinas pró-inflamatórias tais como IL-2, IL-12, interferon-gama (IFN-γ) e fator estimulador de colônias de macrófagos (GM-CSF)[61, 62]. Quando expostos à IL-4, os linfócitos T_{h0} diferenciam-se em linfócitos T_{h2}, os quais medeiam a resposta immune humoral e secretam principalmente citocinas anti-inflamatórias, tais como IL-4, IL-5, IL-10 e IL-13[47, 62]. Mais recentemente, uma nova linhagem de linfócitos T tem sido estudada, os linfócitos T_{h17}, os quais estimulam a secreção principalmente de IL-17 e IL-21. Esta linhagem é produzida quando as células T_{h0} são expostas à IL-21[60]. Já os linfócitos T_{reg} são estimulados pelo fator de transformação do crescimento beta (TGF-β) e secretam tanto TGF-β quanto IL-10, agindo principalmente como uma linhagem anti-inflamatória[47].

Figura 4 – Amadurecimento das células T_0 nos diferentes subgrupos específicos de células T_{h0} (T_{h1}, T_{h2}, T_{h17}) e T_{reg}.



Amadurecimento dos quatro principais subtipos de linfócitos T na doença periodontal: IL-12, IL-4, IL-21 estimulam, respectivamente, o amadurecimento de células T_{h0} nos subtipos T_{h1}, T_{h2}, e T_{h17}, enquanto TGF-β estimula o amadurecimento das células T em T_{reg}. Os subtipos T_{h1} e T_{h17} secretam predominantemente citocinas pró-inflamatórias, enquanto os subtipos T_{h2} e T_{reg} secretam principalmente citocinas anti-inflamatórias. (AFS, 2015)

1.2.4 Como medir a resposta imune na doença periodontal

1.2.4.1 Técnicas de coleta e análise de fluido crevicular gengival (FCG)

O FCG contém grandes quantidades de diferentes componentes moleculares (proteínas, anticorpos), que são derivados do sangue[63-65]. Com o aumento do processo inflamatório gengival, este transudato se transforma em um exsudato presente nas bolsas periodontais e sua nova composição é resultado da presença do biofilme bacteriano e das células dos tecidos periodontais. A presença e volume de FCG podem ser indicativos de mudanças nos tecidos periodontais, uma vez que a quantidade de FCG produzido é aumentada com o aumento da permeabilidade vascular e ulceração do epitélio em sítios inflamados[66, 67]. Desta forma, amostras individuais de FCG podem descrever os eventos inflamatórios que ocorrem

localmente. A coleta de FCG como diagnóstico auxiliar é considerada conveniente por se tratar de um meio não-invasivo, rápido e de execução simples[64, 68].

O FCG pode ser coletado por uma variedade de métodos, tais como sucção, lavagem e absorção[69-71]. O método mais utilizado é o de absorção realizado por meio de tiras de papel absorvente[72, 73]. Nesta técnica, uma tira de papel absorvente é inserida no sulco ou bolsa periodontal, permitindo que o fluido acumule por um período de tempo determinado, sendo sugerido 30 segundos como tempo necessário e suficiente. Imediatamente após a coleta, o volume pode ser mensurado por um aparelho específico (Periotron 8000), baseado em alteração dielétrica da tira de papel[74, 75]. Uma grande limitação desta técnica é que o volume coletado é extremamente baixo, sendo que os filtros de papel utilizados não são capazes de coletar volumes maiores do que 1µl[74]. Sugere-se ainda que é necessário um volume mínimo de fluido coletado para que a análise possa ser realizada com confiabilidade, e este volume é sugerido como sendo de 0.2µl[70].

Após a coleta, rotineiramente é necessário realizar a eluição das proteínas do papel para se determinar a composição do FCG. Para eluição, a técnica de agitação e centrifugação tem apresentado um alto grau de recuperação proteica[70, 76]. Para análise de proteínas do FCG (assim como do sangue, soro ou saliva), diferentes técnicas podem ser empregadas, entre elas destacam-se:

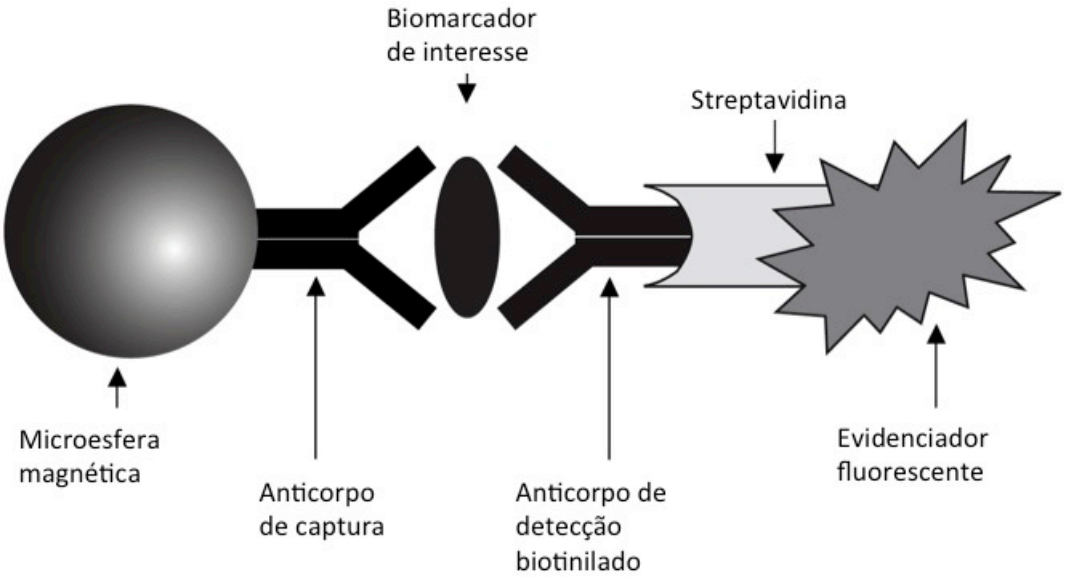
- Citometria de fluxo: detecção óptica-eletrônica de características físicas e/ou químicas de células[77];
- Western Blot: técnica que se utiliza de eletroforese em gel para separar proteínas desnaturadas por massa[58];
- Checkerboard Immunoblotting: quantifica proteínas por quimioluminescência[78];
- ELISA – enzyme-linked immunosorbent assay: teste imunoenzimático que permite a detecção de anticorpos[79, 80];
- Multiplex Assay: análises múltiplas por luminescência, baseada na técnica do ELISA[81].

A técnica mais amplamente utilizada e validada para análise de citocinas é a do ELISA. Nesta técnica um anticorpo de captura fornece a especificidade

imunológica, aonde o antígeno (citocina) é ligado. Em sequencia, um segundo anticorpo ligado a uma enzima de detecção proporciona a amplificação quando ligado ao antígeno. Esta abordagem, conhecida como sanduíche, permite a detecção precisa e sensível do antígeno, neste caso, a citocina de interesse[79, 80]. No entanto, esta técnica permite a análise de apenas uma citocina por alíquota de amostra, dificultando estudos em que análises de múltiplos marcadores são necessárias.

Mais recentemente, uma técnica conhecida como Ensaio Múltiplo (Multiplex Assay) tem sido largamente empregada. Esta técnica é baseada no mesmo princípio de sanduíche do ELISA, no entanto é capaz de analisar vários biomarcadores ao mesmo tempo por luminescência, como na técnica de citometria de fluxo[80, 81]. Para análises múltiplas de proteínas, duas plataformas tem sido desenvolvidas, uma plataforma sólida e uma plataforma de microesferas magnéticas. Na plataforma sólida, o princípio geral é muito similar ao do ELISA, diferindo no número de analitos que podem ser analisados simultaneamente[81]. Já na técnica de microesferas magnéticas, anticorpos de captura específicos são ligados covalentemente a uma microesfera magnética. Este anticorpo reage com o biomarcador de interesse (citocina) e após uma série de lavagens para remover proteínas não ligadas, um anticorpo de detecção biotinilado é adicionado para criar um complexo do tipo sanduíche. O complexo final de detecção é formado com a adição de um indicador fluorescente, a streptavidina (Figura 5). Comparado ao ELISA, o Multiplex Assay é mais sensível, requer menor volume de amostra e permite a medida simultânea de até 100 diferentes proteínas, com menor custo e tempo necessários[82, 83].

Figura 5. Princípio “sanduíche” na técnica de análises múltiplas magnética.



Anticorpos específicos são ligados a uma microesfera magnética. Este anticorpo reage com o biomarcador de interesse (citocina) e um anticorpo de detecção é adicionado para criar um complexo do tipo sanduíche. O complexo final de detecção é formado com a adição de indicadores fluorescentes (adaptado de manual de instruções Bio-Plex Pro Human Chemokine Assay).

1.2.4.2 Biomarcadores da doença periodontal

A maior parte dos estudos realizados em periodontia vale-se de medidas de parâmetros clínicos, como profundidade de sondagem, perda de inserção clínica e sangramento à sondagem, para avaliar progressão de doença. Estes indicadores, contudo, são susceptíveis a erros tanto de medida quanto de interpretação[84, 85]. Além disso, tem sido sugerido que estes parâmetros clínicos tem um valor limitado em predizer progressão de doença[86-88]. Desde a década de 60, pesquisadores vêm investigando bactérias específicas do biofilme subgengival e biomarcadores do fluido crevicular gengival (FCG) como preditores mais precoces e precisos de atividade de doença, como medidas auxiliares de diagnóstico em Periodontia[89].

Biomarcadores podem ser definidos como qualquer biomolécula ou uma característica específica, aspecto e indicador de mudanças em qualquer estrutura biológica que pode objetivamente medir o estado ou situação de um organismo vivo. Em pesquisa clínica, o principal objetivo de analisar um biomarcador é avaliar o estado de saúde de um indivíduo ou o diagnóstico de uma dada doença. Além disso, os biomarcadores podem ser utilizados para avaliar resposta a uma terapia ou tratamento empregado. Desta forma, para serem clinicamente relevantes, estes biomarcadores precisam apresentar tanto sensibilidade (capacidade de classificar

corretamente um indivíduo doente, ou detectar doença quando ela realmente existe) quanto especificidade (capacidade de classificar corretamente um indivíduo como não-doente, ou não detectar doença quando ela não existe)[90, 91].

Existem muitas técnicas laboratoriais que são rotineiramente empregadas em pesquisa de diagnóstico com biomarcadores. Elas fornecem informação de grande valor sobre diferentes sistemas do organismo, incluindo o sistema imune. As técnicas baseadas em anticorpos tem sido utilizadas para se estudar moléculas intra e extracelulares, uma vez que os anticorpos podem ser coletados de diferentes espécies animais[57]. As técnicas mais comuns de pesquisa imunológica em periodontia são os métodos de estudo de proliferação de células T e expressão de citocinas. As principais fontes para análise de biomarcadores da periodontite são o fluido crevicular gengival (FCG), a saliva e o sangue[75].

Além da técnica de coleta de FCG já mencionada anteriormente, a saliva também é apresentada como um meio alternativo comum e não invasivo de coleta de biomarcadores[92], no entanto pode apresentar problemas como alteração no fluxo salivar, ritmo circadiano, tipo de glândula salivar, estímulo salivar, dieta e idade[93]. Além disso, apesar de a saliva apresentar mais sensibilidade, o FCG apresenta maior especificidade[94]. Amostras de soro e sangue também tem sido utilizado em estudos em periodontia[95, 96], no entanto esta técnica tem a desvantagem de ser mais invasiva, e requer treinamento técnico especializado para ser coletado.

1.2.4.3 Validade diagnóstica dos biomarcadores

De um ponto de vista prático, cirurgiões-dentistas clínicos estão interessados na avaliação do risco de sítios que, apesar do tratamento periodontal, continuam mostrando sinais de inflamação periodontal. Neste sentido, sítios identificados como de risco de progressão de doença poderiam receber tratamentos auxiliares, tais como cirúrgico, antibiótico-terapia, ou terapia regenerativa, enquanto sítios com maior tendência a estabilidade poderiam ser mantidos com intervenção minimamente invasiva.

Três diferentes estratégias tem sido utilizadas para investigar o envolvimento e utilidade de citocinas e quimiocinas como biomarcadores de doença. Primeiro, estudos transversais são conduzidos com o objetivo de comparar saúde e doença. Em segundo lugar, estudos clínicos são conduzidos com o objetivo de mostrar se os níveis iniciais de biomarcadores são alterados após terapia periodontal. Por fim,

estudos longitudinais permitem verificar se sítios que apresentam progressão de doença apresentam, também, um diferente perfil de biomarcadores.

Nesse sentido, foi realizada uma revisão sistemática da literatura com metanálise evidenciando os principais biomarcadores estudados em doença periodontal até o ano de 2014 (Artigo 1). Primeiramente, uma busca na literatura evidenciou as 10 citocinas mais estudadas em doença periodontal. Foram selecionados os estudos que apresentavam dados disponível para realização de 2 cálculos de metanálise: estudos transversais, comparando a quantidade de cada uma das 10 citocinas/quimiocinas em pacientes com doença periodontal e saudáveis, e estudos clínicos comparando as quantidades de cada uma das 10 citocinas/quimiocinas antes e após o tratamento da doença periodontal. A análise transversal demonstrou que os níveis das citocinas pró-inflamatórias IL-1β, IL-6 e IFN-γ e a quimiocina CCL2 estão significativamente aumentados em pacientes doentes, quando comparados com pacientes saudáveis. Já as citocinas IL-4, IL-12, IL-17 e a quimiocina IL-8 apresentaram tamanho de efeito moderado e TNF-α e IL-10 apresentaram efeito pequeno e resultados estatísticos inconsistentes. A análise de estudos clínicos evidenciou que os níveis das citocinas pró-inflamatórias IL-1β e IL-17 diminuem significativamente enquanto que o nível da citocina anti-inflamatória IL-4 aumenta significativamente após o tratamento. Nesta análise, não foi observada diferença significativa nos níveis das quimiocinas antes e após o tratamento. Adicionalmente, uma busca por estudos longitudinais também foi realizada, no entanto com quantidade de estudos insuficiente para realizar metanálise. Os resultados desta busca sugerem que sítios com quantidades elevadas de IL-1β, TNF-α, IFN-γ e CCL2 estão mais sujeitos a progressão de doença quando comparados a sítios com baixas quantidades destes biomarcadores.

Desta forma, tendo em vista a atual escassez de estudos analisando o efeito do controle estritamente supragengival durante o período de MPP, e considerando que os marcadores inflamatórios tem um importante papel no desenvolvimento da doença periodontal, este trabalho objetiva realizar uma análise imunológica associada aos resultados clínicos do efeito de dois protocolos de manutenção periodontal empregados em pacientes tratados: controle supragengival realizado pelo binômio paciente/profissional realizado de forma estrita ou associado ao controle profissional subgengival do biofilme em pacientes periodontais tratados.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Comparar o efeito da realização do controle do biofilme supragengival como única forma de intervenção com o controle do biofilme supragengival acrescido do controle profissional do biofilme subgengival nos indicadores imunológicos periodontais presentes no fluido crevicular subgengival de pacientes periodontais tratados ao longo de 12 meses da fase de Manutenção Periódica Preventiva (MPP).

2.2 OBJETIVOS ESPECÍFICOS

a) Identificar as principais citocinas pró- e anti-inflamatórias e quimiocinas que caracterizam a doença periodontal;
b) Comparar o efeito do controle do biofilme supragengival com o efeito do controle dos biofilmes supra e subgengival, realizados pelo binômio paciente-profissional, sobre os marcadores imunológicos (citocinas IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12(p40p70), IL-13, IL-17, GM-CSF, IFN-γ, TNF-α, CCL2, CCL3, CCL4) ao início e aos 3, 6 e 12 meses do período de MPP;
c) Correlacionar variáveis imunológicas e clínicas (presença de biofilme supragengival e inflamação gengival; médias de profundidade de sondagem e perda de inserção clínica; presença de sangramento à sondagem) dos participantes durante a fase de MPP.

3 DESENVOLVIMENTO - ARTIGOS CIENTÍFICOS

3.1 ARTIGO 1: artigo formatado para submissão no Journal of Periodontology.

Gingival crevicular fluid levels of cytokines and chemokines in chronic periodontitis – a systematic review and meta-analysis of human studies.

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Short running title: A meta-analysis on GCF levels of cytokines and chemokines

Key findings: Systematic evidence for a relevant role in ChP is limited to few cytokines and chemokines including IL-1β, IL-4, IL-6, IL-17, and IFN-γ.

ABSTRACT

Background: Chronic periodontitis (ChP) is an inflammatory disease caused by oral biofilms that may lead to destruction of the periodontium, and ultimately tooth loss. This inflammatory process is mediated by cytokines and chemokines produced by the host, which can be detected in gingival crevicular fluid (GCF) and may have diagnostic and prognostic value.

Objectives: to estimate the difference in GCF levels of cytokines/chemokines between periodontally healthy subjects and subjects diagnosed with ChP, to estimate changes in GCF levels of cytokines/chemokines after nonsurgical periodontal treatment, and to establish the predictive value of GCF cytokines/chemokines for disease progression.

Data sources: Studies indexed in MEDLINE/PubMed published in English, Portuguese and Spanish were eligible for this review. A database search up to October 2014 was performed. A manual search of the reference list from review papers and selected articles was also performed.

Review methods: Only studies providing data on GCF levels of cytokines/chemokines in subjects diagnosed with ChP and periondontally healthy controls were included. Cross-sectional, case control, longitudinal and clinical studies were included. Meta-analyses were conducted for those cytokines/chemokines with at least 3 available studies.

Results: Large and significant effect sizes were observed for IL-1β, IL-6 and IFN-γ and MCP-1 indicating that these cytokines/chemokines were at higher GCF levels in subjects diagnosed with ChP than periodontal health. Large and significant decreases in GCF levels of IL-1β and IL-17 after nonsurgical periodontal treatment were observed, whereas an increase was observed for IL-4 following treatment. No conclusions could be drawn with regards to increased risk of disease progression.

Conclusion: Our findings support a relevant role in ChP to a few, mostly pro-inflammatory, cytokines (IL-1β, Il-4, IL-6, IL-17, and IFN-γ) and chemokine (MCP-1). Future studies should be methodologically sound, properly powered, and better described.

Implications of key findings: Future research should focus on longitudinal studies and clinical trials with long-term follow-up, and a better understanding of the role of anti-inflammatory cytokines/chemokines on periodontal stability should be a priority.

Key-words: cytokines, chemokines, periodontal diseases, periodontitis, inflammation.

Chronic periodontitis (ChP) is an inflammatory disease affecting the supporting tissues of the teeth (periodontium), characterized by inflammation of the gingiva and destruction of the periodontal ligament and alveolar bone.[1] It is well established that certain oral bacteria, organized in a biofilm, are the main cause of periodontal disease. Periodontal pathogens stimulate the host immune response to establish an inflammatory reaction that often leads to connective tissue destruction and bone resorption.[2-4] These immune-inflammatory processes are mediated by cytokines and chemokines. Cytokines are soluble proteins produced by various cell types that act as mediators of homeostasis and immunity, and have important housekeeping roles in health and disease.[5-7] Chemokines are signaling proteins produced mainly by myeloid cells such as macrophages that have a crucial role in recruiting specific leukocyte subpopulations to sites of ongoing tissue damage.[6-8] These mediators are produced locally in the periodontal tissues and they can be readily detected in the gingival crevicular fluid (GCF).[9]

Several studies have investigated the role of interleukin-1β (IL-1β), IL-6 and interferon-gamma (IFN-γ) on ChP with conflicting results. Collectively, these pro-inflammatory cytokines appear to contribute to periodontal tissue destruction.[10-15] Few studies have focused on the role of anti-inflammatory mediators for ChP. IL-4 and IL-10 have been investigated as important biomarkers with protective role in alveolar breakdown, i.e., attenuation of disease progression.[10, 16] Among the chemokines, IL-8 has been associated with subclinical inflammation during the initial periodontal lesion, and it has been detected in ChP. [6, 11, 12]

Whereas several narrative reviews have been published on this topic, no attempts to systematic reviews could be identified in the literature. In this context, our aim was to identify cytokines/chemokines with relevant roles in ChP using meta-analytic methods. Our assumptions were that key cytokines/chemokines would be overexpressed in the GCF of subjects diagnosed with ChP than healthy controls; GCF cytokine/chemokine levels would either increase or decrease substantially after periodontal therapy; and, that substantial changes in GCF cytokine/chemokine levels would precede disease progression. Thus, the specific aims of this systematic review and meta-analysis were a) to estimate the difference in GCF levels of cytokines/chemokines between periodontally healthy subjects and subjects diagnosed with ChP using data from cross-sectional and case-control studies, b) to estimate changes in GCF levels of cytokines/chemokines after nonsurgical

periodontal treatment using data derived from clinical studies, c) to establish the predictive value of GCF cytokines/chemokines for disease progression using data from longitudinal studies.

METHODS

The Quality of Reporting of Meta-analyses (QUOROM) statement[17] was followed during the review process and reporting.

Focused Questions

The literature was searched trying to answer the following focused questions according to study design:

a) Cross sectional and case control studies: Can GCF cytokine/chemokine profiles be used to distinguish between periodontal health and ChP?

b) Clinical studies: Do GCF cytokine/chemokine profiles change significantly after non-surgical periodontal treatment?

c) Longitudinal studies: Can GCF cytokine/chemokine profiles be used to predict which periodontal sites are at higher risk of periodontal disease progression, or conversely, at higher likelihood of clinical stability?

Search Strategy

An electronic search of Medline via PubMed database was conducted for articles published in English, Portuguese and Spanish, with no date range limit up to October 2014. The search strategy used the following combination of MeSH terms: (cytokines OR chemokines OR inflammation OR gingival crevicular fluid) AND (periodontal diseases OR periodontitis OR gingivitis). A manual search of the reference list from review papers and selected articles was also performed.

Publication Selection

Titles and abstracts of the identified articles were screened for inclusion. Full-text articles were obtained for potentially relevant studies.

a) Study design selection

Cross-sectional, case control, longitudinal and clinical studies were included.

b) Subjects' characteristics

Only studies including systemically healthy subjects were included. Only data from healthy controls and subjects diagnosed with ChP were used. Data from studies with samples collected from healthy and diseased sites within the same subject were also included. Data derived from subjects/sites with a diagnosis of gingivitis or aggressive periodontitis were not considered.

c) Type of treatments in clinical studies

For clinical studies, only data from nonsurgical periodontal treatment, i.e., subjects treated with scaling and root planning (SRP) were considered for analysis. Data from control groups from clinical studies evaluating local or systemic antibiotics and anti-inflammatory medications were included, i.e., data pertaining to subjects who received only SRP were used.

d) Outcomes

Only studies reporting GCF cytokine/chemokine levels (concentration or amount) around teeth were included. Publications without proper statistical analysis including central tendency measures (means or medians) and variability (standard deviation or data range) were excluded from the analysis.

Data Synthesis

Two reviewers (AFS and PDMA) independently screened titles and abstracts through the databases. Any disagreement was solved by consensus between the reviewers or by a third reviewer (CS). One examiner (AFS) extracted data from the selected studies, and data was reviewed for completeness and accuracy (CS).

Studies characteristics and quality assessment

Studies were summarized in Table 1 according to study design. Study characteristics, case definition, GCF sampling and processing, and risk of bias were described. The risk of bias was assessed by evaluating studies in regards to the following methodological issues: control for age, gender, smoking status; calibration

of the examiners; inclusion or exclusion of systemic involvement and use of medications.

Statistical analysis

Meta-analyses were performed for cytokines/chemokines for which data could be extracted from at least 3 studies. Articles reporting means and standard deviation were included in the meta-analysis. Studies that reported medians, data range and sample size were included, and means and standard deviations were calculated using standard formulas.[18] Studies that presented results in graphic format only were not included. Clinical studies that provided baseline data comparing healthy and diseased groups had the data extracted for both analyses: comparison between groups, and comparison before and after treatment. Data analysis was performed using statistical software (Stata 13.1 for Mac, Stata Corporation, College Station, TX, USA). Fixed and random effects models were used to estimate the standardized mean difference (SMD) and 95% confidence intervals (CI) by the method of Cohen.[19] The heterogeneity of effects among studies was assessed by calculating I^2.[20] Overall estimates for fixed and random models are presented in Tables 2 and 3, and Forest plots are presented in appendix (figures 3 and 4). A sensitivity analysis was carried out to assess influential studies by removing one study at the time.

Heterogeneity was broadly categorized as low, moderate and high following the I^2 statistics cut-off points suggested by Higgins et al.:[20] 25%, 50%, and 75%. Effect sizes were also broadly categorized as small, medium and large following the SMD cut-off points suggested by Cohen:[19] 0.2, 0.5, and 0.8. In order to facilitate the interpretation of the results, we established a graphic summary of the results based on the above described thresholds, significance level, consistency between the fixed and random effect models and taking into consideration the body of available evidence (Fig. 2). Cytokines/chemokines were scored as: three arrows – large (SMD ≥0.8) and significant ($p<0.05$) effect sizes for fixed and random effect models; two arrows – large (SMD ≥0.8) and significant ($p<0.05$) effect size for fixed but not random effect models; one arrow – small/medium (SMD≥0.2 and SMD<0.8) and significant ($p<0.05$) effect size for fixed but not random effect models; the symbol "≈" was used to indicate limited or no effect (SMD <0.2).

RESULTS

The initial search yielded 11,053 publications (Fig. 1). After exclusion of studies based on their titles, 497 abstracts were selected for review, and 138 publications were selected for full text review. Of these, 107 publications were selected including 54 cross-sectional/case control studies, 48 clinical trials and 5 longitudinal studies. Overall, data for 31 cytokines/chemokines were potentially available from these publications, and after an initial assessment 10 cytokines/chemokines fulfilled the eligibility criteria for meta-analysis (Fig. 2). Four studies provided medians without minimum and maximum values,[21-24] one study did not provide estimates for SD,[25] and one study did not report sample size.[26] These studies were not included in the review. A total of 25 cross-sectional/case control studies, 31 clinical studies and 3 longitudinal studies were finally included. Meta-analyses were performed for cross sectional/case-control studies and clinical trials, whereas longitudinal studies results were only described.

IL-1β[27-57] and IL-8[28, 34, 35, 38, 39, 41, 42, 44, 47, 51, 53, 55, 56, 58-64] were the most studied citokines/chemokines with more than 15 studies each. IL-10[38, 40, 53, 55, 65] and IL-12[35, 41, 43, 53, 55, 66] were evaluated in only 5 and 6 studies respectively, and monocyte chemoattractant protein-1 (MCP-1),[35, 41, 51, 61, 67-70] interferon-gama (IFN-γ),[29, 35, 41, 51, 53, 55, 65, 71-73] TNF-α,[38, 40, 41, 48, 51, 53, 55, 57, 74] IL-6,[29, 35, 38, 44, 53, 55-57, 64, 75-78] IL-17,[41, 51, 65, 72, 79-82] and IL-4[35, 40, 41, 44, 53, 55, 71, 72, 83] were studied in an intermediary number of investigations.

Studies characteristics and quality assessment

Table 1 provides a methodological description of the studies included. In general, study samples were small, from convenience population of patients, and not matched or restricted for important confounders. Disease definition varied greatly among studies, but generally involved the presence of attachment loss, deep probing depth and clinical signs of inflammation (bleeding on probing). GCF sampling was mostly done using filter paper strips for 30s; only 9 studies used other sampling methods: 7 used microcapillary tubes, [37, 42, 67-69, 82, 83] and 1 used paper points.[77] Great heterogeneity was observed on the number of sites sampled. Two studies sampled healthy and diseased sites within the same subject.[38, 41] Whereas early studies used ELISA and focused on few cytokines/chemokines, recent studies tended to use Multiplex assay techniques and explored multiple analytes. Nevertheless, 49 out of

59 studies included used ELISA. Inconsistent data reporting was evident with studies reporting concentration and total amounts without a clear trend.

GCF cytokine/chemokine profile in ChP

Table 2 shows the results of meta-analyses comparing subjects with a diagnosis of periodontal health or ChP. A high degree of heterogeneity was observed in all analyses (I^2 > 75%, p<0.001)[20] and the Funnel plots (data not shown) indicated that some studies were at high risk of bias. Nevertheless, the sensitivity analysis did not yield major changes in the estimates; thus, no studies were excluded from the analysis. A comparison between the fixed and random effects models showed major discrepancies in the estimates for IL-12, IL-17, TNF-α and IL-10.

In the fixed effects model, all but one pro-inflammatory cytokines were significantly higher in ChP than periodontal health; IL-12 was lower in disease than health. Among chemokines, subjects diagnosed with ChP had significantly higher IL-8 and MCP-1 than healthy controls. Considering the anti-inflammatory cytokines, IL-4 was significantly lower and IL-10 was significantly higher in periodontal disease than in periodontal health, respectively. In the random effects model, only IL-1β, IFN-γ, IL-6 and MCP-1 remained significant.

Figure 2 summarizes the overall findings. IL-1β, IFN-γ, IL-6, and MCP-1 showed large and significant effect sizes with consistent results in both statistical models. IL-12, IL-17, IL-8, and IL-4 had moderate effect sizes with somewhat inconsistent statistical results. TNF-α and IL-10 had small effect sizes and inconsistent statistical results.

GCF cytokine/chemokine profile changes after nonsurgical periodontal treatment

Table 3 presents the results from meta-analyses comparing GCF cytokine/chemokine levels before and after non-surgical periodontal treatment on subjects diagnosed with ChP. A high degree of heterogeneity was observed for IL-1β, IL-6, IL-10, IL-17, IL-8 and MCP-1 (I^2 > 75%, p<0.001).[20] Heterogeneity was considered moderate for the remaining meta-analyses not achieving significance for IFN-γ and TNF-α. Funnel plots (data not shown) indicated that some studies were at high risk of bias. Nevertheless, the sensitivity analysis did not yield major changes in the estimates.

A comparison between the fixed and random effects models showed major discrepancies in the estimates for IL-6, IL-8 and IL-10. In the fixed effects model, all but two pro-inflammatory cytokines levels decreased significantly after periodontal treatment; no significant changes were observed for IL-12 and TNF-α. Both chemokines, IL-8 and MCP-1, were significantly lower following treatment. Considering the anti-inflammatory cytokines, IL-4 and IL-10 were, respectively, significantly higher and lower after treatment. In the random effects model, only IL-1β, IL-17, and Il-4 remained significant.

Overall, large and significant effect sizes with consistent results were observed for IL-1β, IL-17, and IL-4 (Fig. 2). Moderate effect sizes and some inconsistency in statistical results were observed for MCP-1 and IL-6. IFN-γ, IL-6, IL-8, and IL-10 had small effect sizes and inconsistent statistical results. TNF-α and IL-12 did not change following treatment.

Prediction of disease progression/remission

Few longitudinal studies have assessed the predictive value of cytokines/chemokines for periodontal disease progression, not allowing a meta-analysis to be conducted. Recently, Kinney et al.[84] found that subjects who experienced CAL progression ≥2 mm after 6 months had significantly increased concentrations of IL-1β at baseline when compared to subjects with stable periodontal status. Kinney et al. was the only study that prospectively collected GCF samples. Retrospective evidence was available from 1 study (2 publications) that collected GCF samples only at 2-month follow-up. Silva et al.[85] found that sites having CAL progression >2 mm had significantly higher levels of IL-1β and higher concentration of MCP-1, IL-1β and TNF-α than stable sites at 2 months of follow-up. Dutzan et al.[86] showed that sites having CAL progression >2mm had significantly higher levels of IFN-γ than stable sites at 4 months of follow-up.

DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis, to explore the cytokine and chemokine levels in periodontal health and disease. Pro-inflammatory cytokines IL-1β, IL-6 and IFN-γ, and chemokine MCP-1 were significantly higher in subjects diagnosed with ChP than periodontal health (Fig. 2). Pro-inflammatory cytokines IL-1β and IL-17 significantly decreased, anti-inflammatory

cytokine IL-4 significantly increased after nonsurgical periodontal treatment. No major conclusions could be drawn with regards to increased risk of disease progression other than longitudinal studies appear to support our meta-analyses findings with regards to IL-1β, IFN-γ, and MCP-1.

Can GCF cytokine/chemokine profiles be used to distinguish between health and ChP?

Our results support the observation that pro-inflammatory cytokines IL-1β, IL-6, IFN-γ, and chemokine MCP-1 are significantly higher in individuals diagnosed with ChP than in periodontally healthy controls. The effect size for these citokines/chemokines was considered high (SMD > 0.8) and consistent across statistical models. IL-1β has been long implicated in the pathogenesis of ChP. Its biological effects include increased production of chemokines, expression of prostaglandins and metalloproteinases, enhanced phagocytic activity.[87-89] IL-6 has been characteristically associated with migration of inflammatory cell and osteoclastogenesis.[90] IFN-γ promotes antigen-presenting cell binding and B-cell maturation.[6, 91, 92] Interestingly, the largest effect size was observed for MCP-1, a potent chemoattractant for monocytes, produced by a variety of cell types in response to different signals such as IL-1β and IFN-γ.[8, 93]

Moderate effect sizes and somewhat inconsistent statistical significance were observed for IL-12, IL-17, IL-8 and IL-4. Of these IL-8 and IL-4 had the most consistent results. IL-8 is a chemoattractant factor for polymorphonuclear leukocytes, and is related to a low subclinic inflammatory state of gingivitis and periodontitis.[94] IL-4 is an anti-inflammatory cytokine that inhibits the activity and down-regulates the production of pro-inflammatory cytokines.[6] IL-12 and IL-17 had major changes in their effect sizes when random effect models were used. IL-12 is a pro-inflammatory cytokine that induces IFN-γ, acting in bacterial clearance and protection against periodontal pathogens;[95] therefore, the grater level of this cytokine in health is surprising. IL-17 is a known inducer of RANKL production, which affects osteoclastic bone resorption.[10, 96]

Finally, small effect sizes were observed for TNF-α and IL-10. These findings should be interpreted with caution given the small number of studies with reduced sample sizes for TNF-α, and small number of studies for IL-10. Recent studies have highlighted the importance of TNF-α in activating osteoclasts directly and in synergy

with other cytokines.[97] IL-10 is an anti-inflammatory cytokine that suppresses immune and inflammatory responses, and appears to be involved in the attenuation of periodontal disease.[98]

Do GCF cytokine/chemokine profiles change significantly after nonsurgical periodontal treatment?

Our findings indicate that IL-1β, IL-17 and IL-4 have large and significant changes after nonsurgical treatment. As expected, pro-inflammatory cytokines decreased after treatment, and IL-4, an anti-inflammatory cytokine, increased following treatment. The large, but inconsistent change in GCF levels of MCP-1 following treatment should be interpreted with caution given the limited number of studies and individuals. In perspective, major decreases in MCP-1 levels are likely to occur following treatment.

The observation that several cytokines/chemokines had small or no changes following nonsurgical periodontal treatment is surprising. Whereas the limited number of studies could explain, at least in part, lack of significance for some comparisons, the small changes following treatment question their true effect size and clinical usefulness. This seems to be especially true for IFN-γ and IL-8.

Can cytokine/chemokine profiles be used to predict which periodontal sites are at higher risk of periodontal disease progression, or conversely, at higher likelihood of clinical stability?

Direct evidence of predictive value was only available for IL-1β, which was derived from one short-term study.[84] Sites experiencing CAL progression ≥2 mm over a period of 6 months had significantly higher IL-1β than sites that did not have CAL progression. Whereas this study corroborates the clear trend demonstrated by our meta-analyses, it is important to acknowledge the need for more studies with longer follow-up for the establishment of IL-1β as a predictor of CAL progression.

Indirect evidence was available for IL-1β, TNF-α, IFN-γ and MCP-1, from two short-term studies,[85, 86] which collected data only at follow-up, i.e., retrospective. These cytokines and chemokine were significantly higher in sites experiencing periodontal breakdown at follow-up. Whereas the findings regarding IL-1β and MCP-1 substantiate our meta-analyses results, conflicting and no differences were

observed for IFN-γ and TNF-α, respectively. Collectively, it seems unlikely that these two cytokines are good candidates for predictors of periodontitis progression.

Methodological issues

Strengths of the present study include the systematic selection, evaluation and reporting of the studies, and, for the first time, a statistical estimation of effect size and significance levels for several cytokines and chemokines. Weaknesses are related to lack of original data in some publications and high degree of heterogeneity of the studies including inconsistent inclusion criteria, disease definition, control of important co-factors, GCF sampling methodology, treatment protocols and follow-up intervals. To address these issues we conducted a systematic assessment of the studies and performed a risk of bias assessment, which are both reported herein. Thus, caution should be exercised when interpreting our results. Great discrepancy in reporting was also observed with results presented in total amounts (pg, pg/site, pg/20sec, pg/30sec) or concentrations (pg/ml, pg/μl, ng/ml). In order to address this issue, we calculated the SMD to estimate the effect size instead of the mean difference since this statistics is not influenced by the outcome unit. Moreover, the SMD provides an effect size estimate that can be used to compare among cytokines/chemokines potential biologic impact.

The differences observed between the estimates derived from fixed and random effects models for several cytokines and chemokines warrants discussion. The fixed effect model assumes that all studies are estimating the same effect size, and, consequently, it gives more weight to large studies.[99] In contrast, the random effect model assumes that studies are estimating different effects allowing for differences in study populations such as demographics, disease severity, and other factors to influence the estimates. Thus, smaller studies are given more weight in random than fixed effects models. Whereas the random effect model is appealing from a biological standpoint, larger studies tended to be better described and less prone to bias in our quality assessment. Herein, we chose to provide both estimates for transparency even though it may have complicated understanding for some cytokines/chemokines. In order to improve understating of the results we provided a graphic summary (Fig. 2), which took into consideration effect size and consistency of the results. We weighted large effect size (SMD ≥0.8) and consistency between

the fixed and random effect models to emphasize which cytokines/chemokines prove to be the most promising moving forward.

Future publications should provide better reporting of the study methods and results. Subjects' diagnosis should be based on established periodontal disease classification and case definitions. Important co-factors for periodontitis and local inflammation such as smoking and diabetes should be restricted by the inclusion criteria or explored through stratified analysis of the data when sample sizes permit. GCF sampling should be thoroughly described including clinical parameters of the selected sites (CAL, PPD and BOP), sampling method, time and analysis. To date, paper points for 30 seconds have overwhelmingly been used to collect GCF and it seems a preferred sampling method moving forward. Whether or not samples were pooled for analysis should be clearly stated. The limit of detection (analytical or functional sensitivity) of the assays should be reported, especially if low levels of the analytes are expected or have occurred. Authors are encouraged to report means and SD of the concentration (pg/ml) of the analytes to facilitate future meta-analyses. If a nonparametric analysis is warranted due to data distribution, medians, percentiles and range could be reported in addition to means and SD. Similarly, total amount of protein could be reported in addition to concentration. Finally, future research should take into consideration our findings in that longitudinal studies should be conducted for the cytokines/chemokines with the largest effect size, and cross-sectional/case control studies should be reserved to investigate those with limited amount of evidence.

CONCLUSION

Our findings provide evidence of a relevant role in ChP to a few, mostly pro-inflammatory, cytokines (IL-1β, IL-4, IL-6, IL-17, and IFN-γ) and one chemokine (MCP-1). Longitudinal studies are necessary to further our understanding on these cytokines/chemokines. Future studies should be methodologically sound, properly powered, and better described.

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Figure legends

Figure 1. Flow-chart

Figure 2. Figure 2. Graphic summary of pro- or anti-inflammatory cytokines and chemokines in periodontal health as compared to chronic periodontitis; after treatment as compared to baseline; and in sites with disease progression as compared to stable sites.

Figure 3. Supplementary file 1.: Forest-plots for cytokines and chemokines from studies comparing periodontal health and chronic periodontitis.

Figure 4. Supplementary file 2.: Forest-plots for cytokines and chemokines from studies comparing before and after periodontal treatment.

Table 1: Selected studies

Study characteristics	Case definitions	GCF sampling / processing	Risk of bias
Cross-sectional / Case control studies			
Preiss et al., 1994[27] n=33 (25F/8M) 14H, dental students, 22.8 years 19 CP, patients from dental clinic, 29.3 years	H: not defined CP: at least 8 teeth with PD > 6mm	2 upper teeth per subject, with at least 1 site with PPD ≥5 mm, with paper strips Time of collection not reported Samples pooled for analysis ELISA for IL-1β Results reported in ng/ml	No control for age, gender, smoke Systemic diseases and medications excluded One examiner not calibrated
Mogi et al., 1999[29] n=71 patients from dental clinic 35 H, 48.8 years 17 CP mild, 51.8 years 19 CP severe, 56.3 years	H: PPD <2mm, no CAL, no BOP, no radiographic bone loss CP mild: PPD < 5mm, CAL < 3mm, with BOP CP severe: years, PPD and CAL > 6mm, with BOP	3 to 5 deepest sites, with paper strips for 30s Samples pooled for analysis ELISA for IL-1β, IL-6, TGF- α and IFN-γ Results reported in pg/µl	No control for age, gender, smoke Systemic diseases and medications excluded One examiner not calibrated
Bozcurt et al., 2000[75] n=30 (11F/19M), patients from dental clinic 15 H, 45.67 years 15 CP, 47.13 years	Diagnosis of disease not defined	4 teeth from maxillary anterior area, with paper strips for 3 min Samples analyzed individually ELISA for IL-6 Results reported in pg/µl	No control for gender, smoke Systemic diseases and medications excluded Subjects were matched by age Unclear whether examiners were calibrated or not
Jin et al., 2000[59] n=26 10 H 16 CP, 32 to 55 years	H: PPD <4mm, CAL <1mm CP: PPD ≥5mm and CAL ≥3mm	3 to 4 sites with PPD ≥5mm, CAL ≥3mm and BOP, with paper strips for 30 s Unclear if samples were pooled or not ELISA for IL-8 Results reported in pg/site and pg/µl	No control for age, gender, smoke Systemic diseases and medications excluded One calibrated examiner
Giannopoulou et al., 2003[44] n=40, from private practice 20 H, 38±11 years (13F/7M) 20 CP, 52±8 years (14F/6M)	CAL >5mm in at least 8 sites and radiographic evidence of bone loss	H: one experimental site randomly selected; CP: one deep periodontal site in each quadrant, with filter membrane for 15 s twice in each site Samples pooled for analysis ELISA for IL-1β, IL-4, IL-6 and IL-8 Results reported in pg/30s	No control for age, gender, smoke Systemic diseases and medications excluded One examiner not calibrated
Lee et al., 2003[61] n= 42	H: GI=0, PPD ≤3mm, CAL ≤1mm	Mesiobuccal sites of maxillary premolar-molar teeth, with paper strip for 30 s	No control for age, gender, smoke, Systemic diseases and medications

19 CP, 45.1±10 years		Samples analyzed individually ELISA for IL-1β Results reported in pg/site and ng/ml	
Vernal et al., 2005 [79] n= 24 (17F/7M), patients from dental clinic 8 H, 36.4±7.9 years 16 CP, 38.4±8.2 years	H: absence of CAL or increased pocket depth CP: 5 or more teeth with PPD ≥5 mm and CAL ≥3 mm	2 sites in the periodontally affected sites for CP subjects, and 2 sites from teeth 16 and 26 in H subjects, with paper strips for 30 s. Samples analyzed individually ELISA for IL-17 Results reported in pg, pg/µl and pg/µg	No control for age, gender Systemic diseases, medications and smokers excluded One calibrated examiner
Biyikoglu et al., 2006[32] Kardesler et al., 2008[33] n= 34 (22F/12M) 17 H, dental school staff, 40.65±6.7 years 17 CP, dental school clinic, 49.12±6.64 years	Diagnosis criteria according Armitage, 1999	2 inflamed sites from single-rooted teeth, with paper strips for 30 s Samples pooled for analysis ELISA for IL-1β Results reported in pg and pg/µl	No control for age, gender, smoke Systemic diseases, medications excluded Unclear whether examiners were calibrated or not
Yucel et al., 2008[43] n=26, from dental school clinic 14 H, 35.6 years (9F/5M) 12 CP, 37.8 years (7F/5M)	H: PPD ≤3mm, CAL ≤1mm CP: PPD ≥5mm and bone loss ≥25% at least in 6 sites.	6 maxillary anterior sites with PPD ≥5mm and bone loss ≥25%, with paper strips for 30 s Samples pooled for analysis ELISA for IL-1β and IL-12 Results reported in pg and pg/µl	No control for age, gender Systemic diseases, medications, smokers excluded One examiner not calibrated
Perozini et al., 2010[36] n=36 (25F/11M), patients from dental school clinic, 18-60 years 12 H, 23.75±3.22 years 12 CP 43.75±11.50 years	Diagnosis criteria according Armitage, 1999	2 randomly selected teeth, with paper strips for 30 s Samples analyzed individually ELISA for IL-1β Results reported in pg/site and pg/µl	No control for age, gender, smoke Systemic diseases, medications excluded Unclear whether examiners were calibrated or not
Teles et al., 2010 [34] n= 40 (25F/15M), patients from dental clinic 20 H, 38±13 years 20 CP, 55±12 years	H: PPD <3 mm, CAL <2 mm CP: 8 sites with PPD ≥4 mm and CAL ≥2 mm	Mesio-buccal aspect of all teeth, with paper strips for 30 s Samples analyzed individually Checkerboard immunoblotting for IL-1β and IL-8 Results reported in pg/site	No control for age, gender, smoke Systemic diseases, medications excluded One calibrated examiner
Chaudhari et al., 2011[37] n= 60 30 H 30 CP	Diagnosis criteria according periodontal disease index by Ramfjord	Any 1 of the maxillary anterior teeth with microcapillary tubes (20 µl) Samples analyzed individually ELISA for IL-1β Results reported in pg/ml	No control for age, gender Systemic diseases, medications, smokers excluded Unclear whether examiners were calibrated or not
Haba et al., 2011[77] n= 90 (43F/42M) 40 H 50 CP	Diagnosis of disease not defined	The most damaged sites, with paper point for 30s Samples analyzed individually ELISA for IL-6 Results reported in pg/ml	No control for age, gender, smoke, systemic diseases, medications Three examiner not calibrated
Fujita et al., 2012[38]	H: sites with PPD ≤3 mm and	One diseased and one healthy site, with paper strips for 30 s	Matched for age, gender

n= 50 (27F/23M) 50 CP, from dental clinic, 59.8±8.5 years	no BOP CP: sites with PPD ≥5mm, CAL≥3mm and BOP	collected 3 times Samples pooled for analysis Luminex assay for IL-1β, IL-6, IL-8, and TNF-α Results reported in pg	Systemic diseases, medications, smokers excluded Unclear whether examiners were calibrated or not
Shaker et al., 2012[80] n= 40 (20F/20M), patients from dental clinic 15 H, 25±2.58 years 25 CP, 40.2±2.65 years	Diagnosis criteria according Armitage, 1999	Site with deepest periodontal pocket in each quarter with paper strips for 30 s Unclear if samples were pooled or not ELISA for IL-17 Results reported in pg and pg/µl	No control for age, gender Systemic diseases, medications, smokers excluded One calibrated examiner
Anil et al., 2013 [69] n= 60 (60M) 30 H, staff and students, 34.53±6.19 years 30 CP, dental clinic, 34.57±7.76 years	Diagnosis criteria according Armitage, 1999	Interproximal surfaces from teeth 3, 9, 19 and 25 with microcappilary pipette (1µl) Unclear if samples were pooled or not ELISA for MCP-1 Results reported in pg/µl	No control for age, gender Systemic diseases, medications, smokers excluded One calibrated examiner
Cetinkaya et al., 2013 [40] n= 32 (14F/18M) patients from dental clinic 16 H, 28.06±6.18 years 16 CP, 44±7 years	Diagnosis criteria according Armitage, 1999	Mesio-buccal and distal-buccal surfaces from maxillary anterior teeth with paper strips for 30 s Unclear if samples were pooled or not ELISA for IL-1β, TNF-α, and IL-4 Results reported in pg and pg/µl	No control for age, gender Systemic diseases, medications, smokers excluded Unclear whether examiners were calibrated or not
Lagdive et al., 2013 [62] n= 80 (32F/48M), patients from dental clinic 20 H 20 CP Mild 20 CP Moderate 20 CP Severe	Diagnosis criteria according to Ramfjord, 1959	1 site (maxillary bucal) with paper strips for 30 s Samples analyzed individually ELISA for IL-8 Results reported in pg/µl	No control for age, gender, smoke Systemic diseases, medications excluded Unclear whether examiners were calibrated or not
Shimada et al., 2013 [41] n= 11 11 CP, dental clinic	Diagnosis criteria according to Armitage, 1999	One healthy (PD <3 mm) and 2 diseased sites (PD 5-6 mm), 4 times for each site, with paper strips for 30 s Unclear if samples were pooled or not Multiplex for IL-1β IL-8, IL-4, TNF-α, IL-17, IFN-γ and MCP-1 Results reported in pg/site	Groups matched by age and gender Systemic disease, medications and smokers excluded One examiner not calibrated
Haytural et al., 2014[70] n= 20 (8F/12M) 10 H, 33.1±4 years 10 CP, 41.6±4.8 years	H: PPD and CAL ≤3mm and BOP at ≤10% of sites. CP: at least 20 sites with PPD ≥5mm and CAL ≥4mm	CP: 2 sites with PPD ≥5mm; H: 2 sites with no pocket or bleeding with paper strips for 30 s Samples analyzed individually Multiplex for MCP-1 Results reported in total amounts	Groups matched by age and gender Systemic disease, medications and smokers excluded Unclear whether examiners were calibrated or not
Jacob et al., 2014[42] n= 30 (9F/21M), patients from rural area 15 H, 28.3±6.4 years 15 CP, 42.9±11.6 years	Diagnosis criteria according to Armitage, 1999	5 non-adjacents sites with the greatest PPD with microcapillary pipette (3µl) Samples pooled for analysis ELISA for IL-1β and IL-8 Results reported in pg/µl	No control for age, gender Systemic diseases, medications, smokers excluded One calibrated examiner
Keles et al., 2014 [78]	H: GI =0; PPD <3 mm, BOP-,	Site with highest clinical signals of inflammation (redness and	Groups matched by age and gender

n= 30 (15F/15M), patients from dental clinic 15 H, 38.07±4.77 years 15 CP, 42.6±7.98 years	no CAL CP: GI >2, PPD ≥4 mm, BOP+, CAL ≥5 mm	swelling and radiographic bone loss) with paper strips for 30 s Samples analyzed individually ELISA for IL-6 Results reported in pg/sample and pg/ml	Systemic disease, medications and smokers excluded One examiner calibrated
Papathanasiou et al., 2014[73] n= 25 (14F/11M), patients from dental clinic 14 H, 26.3±2.6 years 11 CP, 49.2±14,9 years	H: BOP <20%, CAL <3mm, no radiographic bone loss CP: >6 teeth with >1 site with PPD >5mm, CAL >3mm, BOP and radiographic bone loss	Sampling sites not defined wit paper strips for 30 s Samples analyzed individually Multiplex for IFN-γ and IL-4 Results reported in pg/site	No control for age, gender Systemic diseases, medications, smokers excluded One examiner not calibrated
Single-arm clinical trial (before and after SRP, no comparison group, only chronic periodontitis subjects)			
Tsai et al., 1995[28] n= 16 (6F/10M), 39.8 years Follow-up of 1 month	Diagnosis of disease not defined	Disto-buccal sites of randomly selected teeth with paper strips for 30s Samples analyzed individually ELISA for IL-1β and IL-8 Results reported in pg/site and ng/ml	Systemic disease, medications excluded Smokers included Unclear whether examiners were calibrated or not
Alexander et al., 1996[45] n= 15 (5F/10M), 28 to 67 years Follow-up of 1 month	Diagnosis of disease not defined	All mesio- and disto-lingual sites from the most distal molar to the distal surface of the canine, and the mid-buccal site of the first and second molars, 2 samples per site, with paper strips, for 30 to 60 s Unclear if samples were pooled or not ELISA for IL-1β Results reported in pg and pg/ml	Systemic disease, medications excluded Smokers included Unclear whether examiners were calibrated or not
Chung et al., 1997[58] n= 30 (?F/?M), 41.0±1.9 years Follow-up of 2 weeks	At least 4 sites with CAL ≥ 4mm and PPD ≥ 3mm.	Mesio-lingual of all bicuspid teeth with papers strips for 30s Unclear if samples were pooled or not ELISA for IL-8 Results reported in pg and pg/μl	Systemic disease, medications excluded Smokers included Unclear whether examiners were calibrated or not
Gamonal et al., 2000, 2001[47, 63] n= 12 (8F/4M), dental clinic, 47.16±11 years Follow-up of 2 months	5-6 teeth with PPD >6 mm and CAL >3 mm	6 sites per subject (2 sites with PPD ≤3 mm, 2 sites with PPD 4-6mm, 2 sites with PPD ≥6 mm) with paper strips for 30s Samples analyzed individually ELISA for IL-1β, IL-8 and IL-10 Results reported in pg and pg/μl	Systemic disease, medications excluded Smokers included Unclear whether examiners were calibrated or not
Tuter et al., 2001[30] n= 25 (11F/14M), dental clinic, 47.2 years Follow-up of 6 weeks	PPD and CAL ≥5mm and radiographic bone loss	The most severely affected upper anterior sextant with paper strips for 30 s Unclear if samples were pooled or not ELISA for IL-1β Results reported in pg/site and pg/μl	Systemic disease, medications excluded Smokers included Unclear whether examiners were calibrated or not
Jin et al., 2002[60] n= 16, 32-55 years Follow-up of 4 weeks	PPD ≥5mm, CAL ≥3mm, radiographic bone loss on at least 2 teeth per quadrant	3-4 sites with deepest PPD with paper strips for 30s Unclear if samples were pooled or not ELISA for IL-8 Results reported in pg/site and pg/μl	Systemic disease, medications excluded Smokers included One calibrated examiner

Navarro-Sanchez et al., 2007[48] n= 10 (7F/3M), dental clinic, 56.4 years Follow-up of 6 months	Diagnosis criteria according to Armitage, 1999	3 deepest site in different quadrants with paper strips for 30s Samples analyzed individually ELISA for IL-1β and TNF-α Results reported in pg/µl	Systemic disease, medications excluded Smokers included One calibrated examiner
Tsai et al., 2007 [71] n= 17 (6F/11M), dental clinic, 32-64 years) Follow-up of 1 month	Diagnosis of disease not defined	Mesiobuccal surfaces of maxillary teeth with PPD and CAL 4-10 mm with paper strips for 30s Samples analyzed individually ELISA for IL-4 and IFN-γ Results reported in pg and pg/µl	Systemic disease, medications excluded Smokers included Unclear whether examiners were calibrated or not
Pradeep et al., 2008,[83] 2009(1),[68] 2009(2),[67] n= 20, 33.95±3.07 years Follow-up of 6-8 weeks	GI >1, CAL ≥1 mm, PPD ≥4 mm	1 site per subject with greater clinical signal of inflammation (redness, bleeding on probing and edema) and highest CAL with microcapillary pipette (1µ) Samples analyzed individually ELISA for IL-4, IL-17, MCP-1 Results reported in pg/µl	Systemic disease, medications, smokers excluded One examiner not calibrated
Buduneli et al., 2009[81] n= 10 (5F/5M), dental clinic, 37 to 55 years Follow-up of 4 weeks	2 interproximal sites with PPD ≥5mm, CAL ≥4mm and radiographic bone loss	4 interproximal sites with with PPD ≥5mm, CAL ≥4mm and BOP with paper strips for 30s Samples pooled in 2 for analysis ELISA for IL-17 Results reported in pg/2 samples and pg/µl	Systemic disease, medications, smokers excluded Unclear whether examiners were calibrated or not
Thunell et al., 2010 [35] n= 6, 40 to 75 years Follow-up of 6-8 weeks	1/3 of dentition with CAL ≥5 mm	4 periodontally diseased (PPD and CAL ≥5 mm, BOP+) sites and 2 healthy sites (PPD and CAL ≤3 mm, BOP-) wit paper strips for 30s Unclear if samples were pooled or not Multiplex for IL-1β, IL-6, IL-8, IFN-γ, IL-4 and MCP-1 Results reported in pg/30sec	Systemic disease, medications, smokers excluded Unclear whether examiners were calibrated or not
Zhao et al., 2011 [72] n= 30, (11F/19M), dental clinic, 44 years Follow-up of 6 weeks	2 teeth per quadrant with PPD ≥5 mm and CAL ≥3 mm	1 molar teeth with PPD 3-8 mm with paper strips for 30s Samples analyzed individually ELISA for IL-17, IL-4 and IFN-γ Results reported in ng/ml	Systemic disease, medications, smokers excluded One examiner calibrated
Fentoglu et al., 2012 n= 28 (14F/14M), 31-54 years, dental clinic Follow-up of 3 months	Diagnosis criteria according to Armitage, 1999	3 deepest sites from 1 molar, 1 premolar and 1 canine, with paper strips for 30s Unclear if samples were pooled or not ELISA for IL-6, IL-8 and TNF-α Results reported in pg/ml	Systemic disease, medications, smokers excluded One examiner calibrated
Konopka et al., 2012 [39] n= 30 (16F/14M), dental clinic, 48.7±9.1 years Follow-up of 4 weeks	Diagnosis criteria according to Armitage, 1999	mesiobuccal sites with paper strips for 30s Unclear if samples were pooled or not ELISA for IL-1β and IL-8 Results reported in pg/sample	Systemic disease, medications, smokers excluded One examiner not calibrated
Toker et al., 2012 [52] n= 30, (8F/7M), 38.7±5.9 years	>30% sites with CAL and PPD ≥5 mm	Sites sampling not defined with paper strips for 30s Unclear if samples were pooled or not ELISA for IL-1β, IL-17 and IFN-γ	Systemic disease, medications, smokers excluded One examiner not calibrated

Follow-up of 6 weeks		Results reported in pg/site	
Fu et al., 2013 [65] n= 148 (72F/76M), 43.8±5.51 years Follow-up of 6 months	Diagnosis: not defined	4 periodontal sites from 4 different quadrants with paper strips for 30s Unclear if samples were pooled or not ELISA for IL-17 and IFN-γ Results reported in pg/ml	Systemic disease, medications, smokers excluded Unclear whether examiners were calibrated or not
Randomized Clinical Trials (only chronic periodontitis, only groups with SRP as treatment)			
Liu et al., 1999[46] n=8 (?F/?M), dental clinic Split-mouth design Follow-up of 12 weeks SRP vs. PDT vs. SRP + PDT 6 weeks later vs. PDT + SRP 6 weeks later	Radiographic evidence of bone loss, PPD 4-6mm and BOP	Middle of the 3 adjacent single-root teeth in each quadrant with paper strips for 30 s Samples analyzed individually ELISA for IL-1β Results reported in pg/ml	No data from systemic disease, smoke Unclear whether examiners were calibrated or not
Lui et al., 2011[49] n=24 (14F/10M), 50 years Split-mouth design Follow-up of 1 month SRP vs. SRP + PDT	At least 2 single-rooted teeth on each side of the mouth having PPD ≥5 mm, interproximal CAL ≥3 mm and radiographic bone loss	2 sites from a single-rooted teeth with PPD ≥5 mm, interproximal CAL ≥3 mm with paper strips for 30 s Samples pooled for analysis ELISA for IL-1β Results reported in pg/ml	No control for age, gender, smoke Systemic disease, medications excluded One examiner not calibrated
Eltas et al., 2012 [50] n= 20 (10F/10M), dental clinic, 46.1±8.3 years Split-mouth design Follow-up for 9 months SRP vs. SRP + PDT	3 or more teeth having at least two quadrants with PPD between 4 and 6mm and radiographic bone loss	1 site per group with PPD 4-6 mm with paper strips for 30s Samples analyzed individually ELISA for IL-1β There is no report of the measurement unit	No control for age, gender, smoke, Systemic diseases and medications excluded One calibrated examiner
Giannopoulou et al., 2012 [51] n= 32 (9F, 23M), dental clinic, 52 years Split-mouth design Follow-up of 6 months SRP vs. DSL vs. PDT	≥1 site in each quadrant with PPD ≥5 mm, CAL ≥2 mm and BOP+	1 deepest pocket from each quadrant with membrane strips for 20s Samples analyzed individually Multiplex for IL-1β, IL-8, IL-17, IFN-γ, MCP-1, and TNF-α Results reported in pg/20sec	No control for age, gender, smoke, Systemic diseases and medications excluded Unclear whether examiners were calibrated or not
Goutoudi et al., 2012[64] n=12, dental clinic, Split-mouth design Follow-up of 32 weeks SRP vs. SRP + Widman flap	Multiple sites with PPD ≥5mm and GI 2 or 3	3 sites with PPD ≥ 5mm with paper strips for 30s Samples analyzed individually ELISA for IL-6 and IL-8 Results reported in pg/µl	No control for age, gender, smoke, Systemic diseases and medications excluded Unclear whether examiners were calibrated or not
Luchesi et al., 2013 [53] n= 37 (10F/6M), dental clinic, 50.75±8.18 years Follow-up of 6 months SRP vs. SRP + PDT	Diagnosis criteria according to Armitage, 1999, and one class II furcation	Furcation class II sites with paper strips for 15s Unclear if samples were pooled or not Multiplex for IFN-γ, IL-1β, IL-4, IL-6, IL-8 and TNF-α Results reported in pg/ml	No control for age, gender Systemic diseases, medications and smokers excluded One calibrated examiner
Queiroz et al., 2013 [54]	Diagnosis criteria according	1 site each group with PPD ≥5 mm with paper strips for 30s	No control for age, gender, smoke,

n= 20 (11F/9M), dental clinic, 46.05±6.38 years Split-mouth design Follow-up of 3 months SRP vs. SRP + PDT	to Armitage, 1999	Samples analyzed individually ELISA for IL-1β Results reported in pg/µl	Systemic diseases and medications excluded One calibrated examiner
Kolbe et al., 2014 [55] n= 22 (11F/9M), dental clinic, 48.52±11.71 years Split-mouth design Follow-up of 6 months SRP vs. PDT vs. photosensitizer	At least 3 single-rooted teeth with PPD ≥5 mm and BOP+	teeth with class II furcation with PPD ≥5 mm and BOP+ with paper strips for 30s Unclear if samples were pooled or not Multiplex for IL-1β, IL-6, IL-4, IFN-γ, IL-8, TNF-α Results reported in pg/ml	No control for age, gender Systemic diseases, medications and smokers excluded One calibrated examiner
Ozgoren et al., 2014[74] n= 32 (7F/9M), dental clinical, 42.3±7.3 years Follow-up of 30 days SRP vs. SRP + non steroidal anti-inflammatory	At least 4 sites with PPD 4-6mm and radiographic bone loss	4 upper incisors with paper strips for 30s Samples pooled for analysis ELISA for TNF-α Results reported in pg	Subjects matched for age, gender Systemic diseases, medications and smokers excluded One calibrated examiner
Saglam et al., 2014[56] n= 30 (12F/18M), dental clinic, 42.13±9.05 Follow-up of 6 months SRP vs. SRP + PDT	Diagnosis criteria according to Armitage, 1999	3 deepest pockets with PPD ≥5 mm with paper strips for 30s Samples pooled for analysis ELISA for IL-1β, IL-8 and IL-6 Results reported in pg/30sec	No control for age, gender Systemic diseases, medications and smokers excluded One examiner not calibrated
Longitudinal Studies			
Silva et al., 2008[85], Dutzan et al., 2009[86] n= 18 (13F/5M), dental clinic, 45.66±6.67 Follow-up of 2 months	Disease: at least 6 teeth had sites with PPD ≥ 5mm, CAL ≥ 3mm and radiographic bone loss Disease progression: CAL≥2mm in 2 sites	1 site with disease progression and 1 site without disease progression, with paper strips for 30s Samples analyzed individually ELISA for IL-1β, TNF-α, IFN-γ and MCP-1 Results reported in pg and pg/µl	No control for age, gender, smokers Systemic diseases, medications excluded One calibrated examiner Cytokines/Chemokines assessed only at 2 months follow-up.
Kiney et al., 2014[84] n=83, dental clinic Follow-up of 6 months	Disease: at least 4 sites with PPD > 4mm and CAL > 3mm Disease progression: CAL > 2mm	Mesiobuccal aspect of 8 sites with deepest PPD, with paper strips for 30s Samples pooled for analysis Quantibody for IL-1β Results reported in pg/ml	No control for age, gender, smokers Systemic diseases, medications excluded Three calibrated examiners Cytokines/chemokines assessed at baseline and 6 months follow-up

BOP - bleeding on probing, CAL - clinical attachment loss, PPD – pocket probe depth, H – health, CP – chronic periodontitis, SDD - subantimicrobial-dose doxycycline, PDT – photodynamic therapy, DSL – subgingival irradiation

Table 2: Standardized mean differences between periodontal health and periodontitis for selected cytokines and chemokines (fixed and random effects). Data derived from cross-sectional and case control studies.

Group	Cytokine/ Chemokine	Number of studies	Sample size		Heterogeneity		Standardized mean difference*					
							Fixed effects			Random effects		
			Health	ChP	I^2 (%)	X^2	Estimate*	95% CI	p-value	Estimate*	95% CI	p-value
Pro-inflammatory												
IL-1 family	IL-1β	15	342	373	87.0	<0.001	1.36	1.19, 1.54	<0.001	1.43	0.93, 1.92	<0.001
T_{h1}	IFN-γ	5	198	206	80.8	<0.001	0.72	0.51, 0.92	<0.001	1.03	0.36, 1.70	0.003
	IL-12	3	65	68	96.2	<0.001	-0.97	-1.40, -0.53	<0.001	-0.49	-2.74, 1.76	0.669
T_{h2}	IL-6	6	181	175	93.1	<0.001	0.80	0.57, 1.03	<0.001	1.64	0.66, 2.63	0.001
T_{h17}	IL-17	4	166	211	97.3	<0.001	1.47	1.22, 1.72	<0.001	0.32	-1.65, 2.29	0.750
TNF family	TNF-α	3	77	88	94.8	<0.001	0.35	0.02, 0.68	0.037	0.27	-1.45, 2.00	0.755
Chemo-kines	IL-8	12	226	327	94.6	<0.001	0.76	0.55, 0.96	<0.001	0.70	-0.21, 1.62	0.132
	MCP-1	7	89	142	96.5	<0.001	1.57	1.18, 1.97	<0.001	3.12	0.92, 5.31	0.005
Anti-inflammatory												
T_{h2}	IL-4	4	79	102	91.8	<0.001	-0.93	-1.27, -0.59	<0.001	-1.01	-2.20, 0.18	0.095
T_{h2}/T_{reg}	IL-10	3	198	214	94.3	<0.001	0.54	0.34, 0.75	<0.001	-0.07	-1.11, 0.97	0.896

* positive estimates indicate higher cytokine/chemokine levels for periodontitis than health; negative estimates indicate higher cytokine/chemokine levels for health than periodontitis.
ChP – chronic periodontitis

Table 3: Standardized mean difference following periodontal treatment for selected cytokines and chemokines (fixed and random effects). Data derived from clinical studies.

Group	Cytokine Chemokine	Number of studies	Sample size		Heterogeneity		Standardized mean difference*					
							Fixed effects			Random effects		
			Before	After	I^2 (%)	X^2	Estimate*	95% CI	p-value	Estimate*	95% CI	p-value
Pro-inflammatory												
IL-1 family	IL-1β	13	347	346	84.7	<0.001	0.70	0.54, 0.87	<0.001	0.85	0.43, 1.27	<0.001
T_{h1}	IFN-γ	7	263	262	43.5	0.101	0.21	0.036, 0.38	0.018	0.20	-0.08, 0.47	0.166
	IL-12	3	49	48	68.3	0.043	-0.12	-0.53, 0.28	0.545	0.01	-0.76, 0.79	0.973
T_{h2}	IL-6	6	136	135	91.8	<0.001	0.35	0.09, 0.61	0.007	0.12	-0.81, 1.06	0.794
T_{h17}	IL-17	4	207	207	88.6	<0.001	1.22	1.01, 1.44	<0.001	0.81	0.01, 1.61	0.048
TNF family	TNF-α	5	129	128	50.0	0.091	-0.01	-0.29, 0.27	0.931	0.04	-0.37, 0.45	0.844
Chemo-kines	IL-8	10	227	226	89.4	<0.001	0.21	0.01, 0.41	0.036	0.14	-0.48, 0.77	0.648
	MCP-1	3	58	58	95.1	<0.001	0.94	0.52, 1.37	<0.001	1.82	-0.61, 4.25	0.143
Anti-inflammatory												
T_{h2}	IL-4	6	116	115	70.4	0.005	-0.86	-1.13, -0.58	<0.001	-0.81	-1.33, -0.29	0.002
T_{h2}/T_{reg}	IL-10	3	191	190	89.4	<0.001	0.41	0.20, 0.61	<0.001	-0.02	-0.86, 0.83	0.969

* positive estimates indicate higher cytokine/chemokine levels before treatment than after; negative estimates indicate higher cytokine/chemokine levels after treatment than before.

3.2 Figuras referentes o artigo 1

Figure 1.

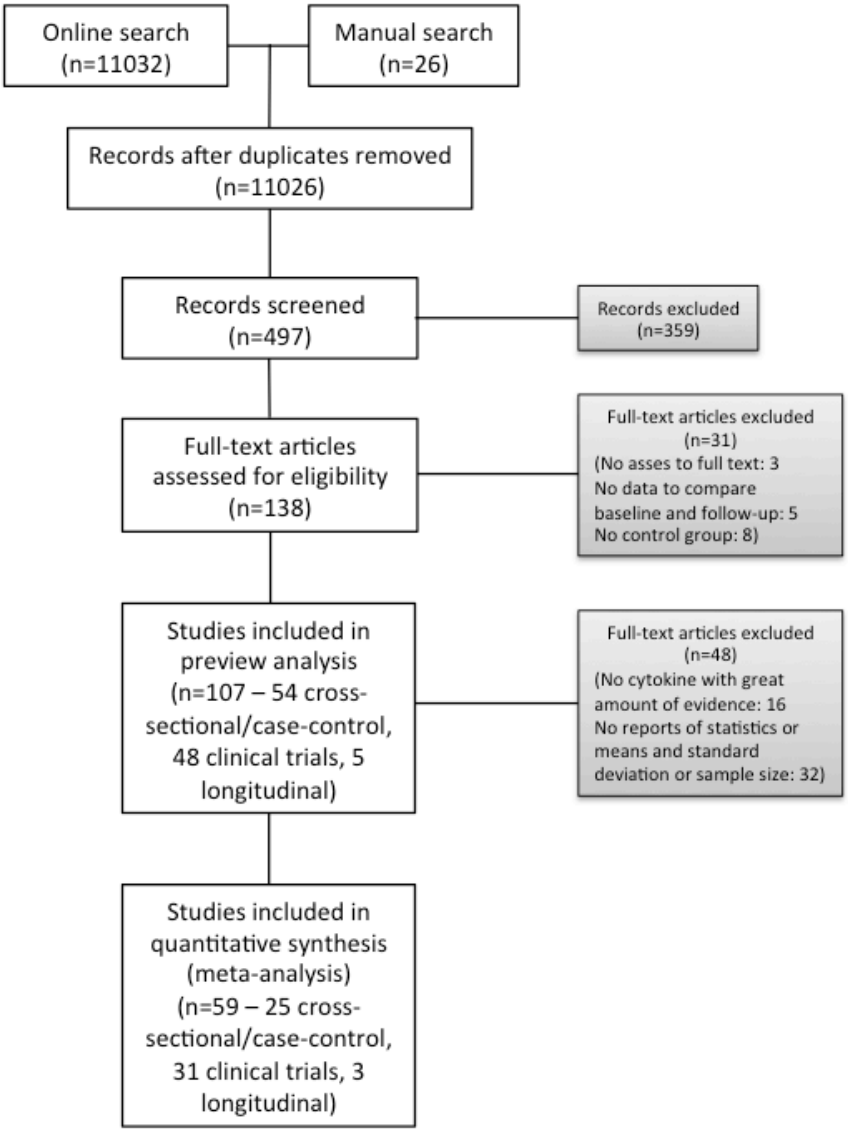


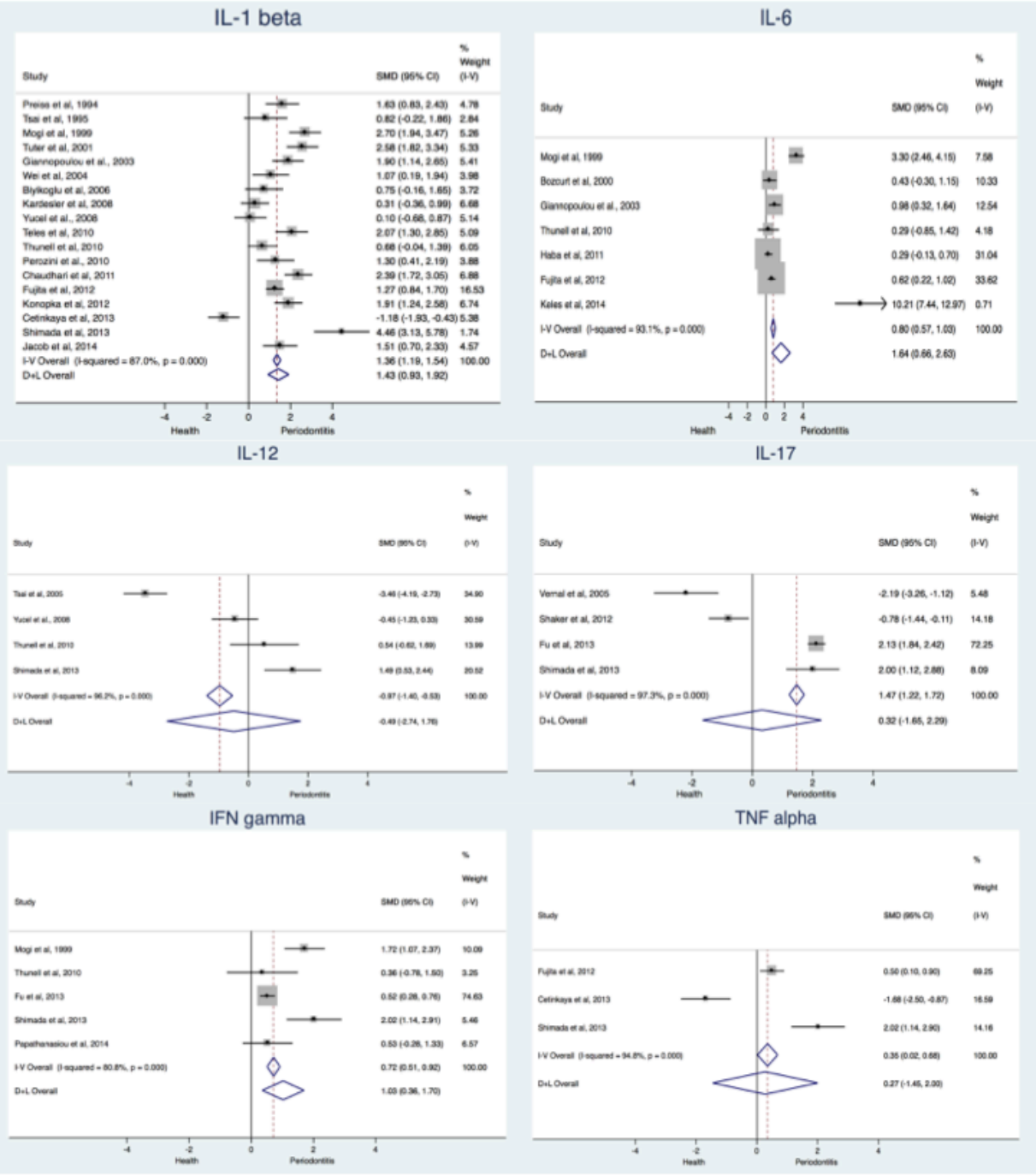
Figure 2.

Group	Cytokine / chemokine	Main Source	Main function	Presence in ChP Vs. periodontal health	Changes after treatment	Risk of disease progression
Pro-inflammatory cytokines/chemokines						
IL-1 family	IL-1β	MØ, EC, fibroblasts, DC	Inflammatory cell migration, osteoclastogenesis	↑↑↑	↓↓↓	↑
T_{h1}	IFN-γ	T cells and natural killers cells	Differentiation of T_0 into T_{h1}, stimulation of MØ, osteoclastogenesis.	↑↑↑	↓	↑
	IL-12	MØ, DC	Enhance T_0 differentiation in T_{h1} and NK proliferation	↓↓	≈	?
T_{h2}	IL-6	MØ, EC, T cells	Inflammatory cell migration, osteoclastogenesis	↑↑↑	↓	?
T_{h17}	IL-17	T cells	Stimulates TNF-α, IL-6 and IL-1β production, enhance RANKL expression	↑↑	↓↓↓	?
TNF family	TNF-α	MØ, T cells	PMN migration, up-regulates IL-1β, IL-6 and RANKL expression	↑	≈	↑
Chemo-kines	IL-8	MØ and EC	Osteoclast differentiation and activity; attract PMN to the inflammation site	↑↑	↓	?
	CCL-2 (MCP-1)	MØ, EC, T cells	PMN recruitment, chemo attractant for monocytes	↑↑↑	↓↓	↑
Anti-inflammatory cytokines						
T_{h2}	IL-4	T (CD4+), mast cells	T_0 proliferation, IL-10 production, inhibits pro-inflammatory cytokines activity, down-regulates IL-1β, IL-6, TNF-α and T_{h1} cells production	↓↓	↑↑↑	?
T_{h2}/T_{reg}	IL-10	MØ, T_{reg}	Inhibits MØ antigen-presenting capacity, activate OPG	↑	↓	?

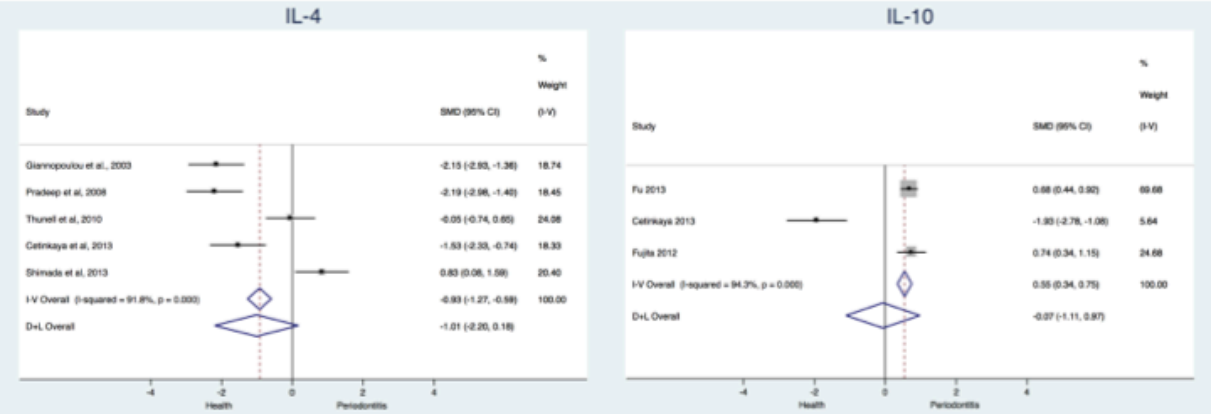
Three arrows – three arrows – large (SMD ≥0.8) and significant ($p<0.05$) effect sizes for fixed and random effect models; two arrows – large (SMD ≥0.8) and significant ($p<0.05$) effect size for fixed but not random effect models; one arrow – small/medium (SMD≥0.2 and SMD<0.8) and significant ($p<0.05$) effect size for fixed but not random effect models; the symbol "≈" was used to indicate limited or no effect (SMD <0.2); the question mark was used to identify citokines/chemokines without estimates.. ChP: chronic periodontitis; MØ: macrophages; EC: epithelial cells; DC: dendritic cells.

Figure 3. Supplementary file 1.

3a. Pro-Inflammatory Cytokines

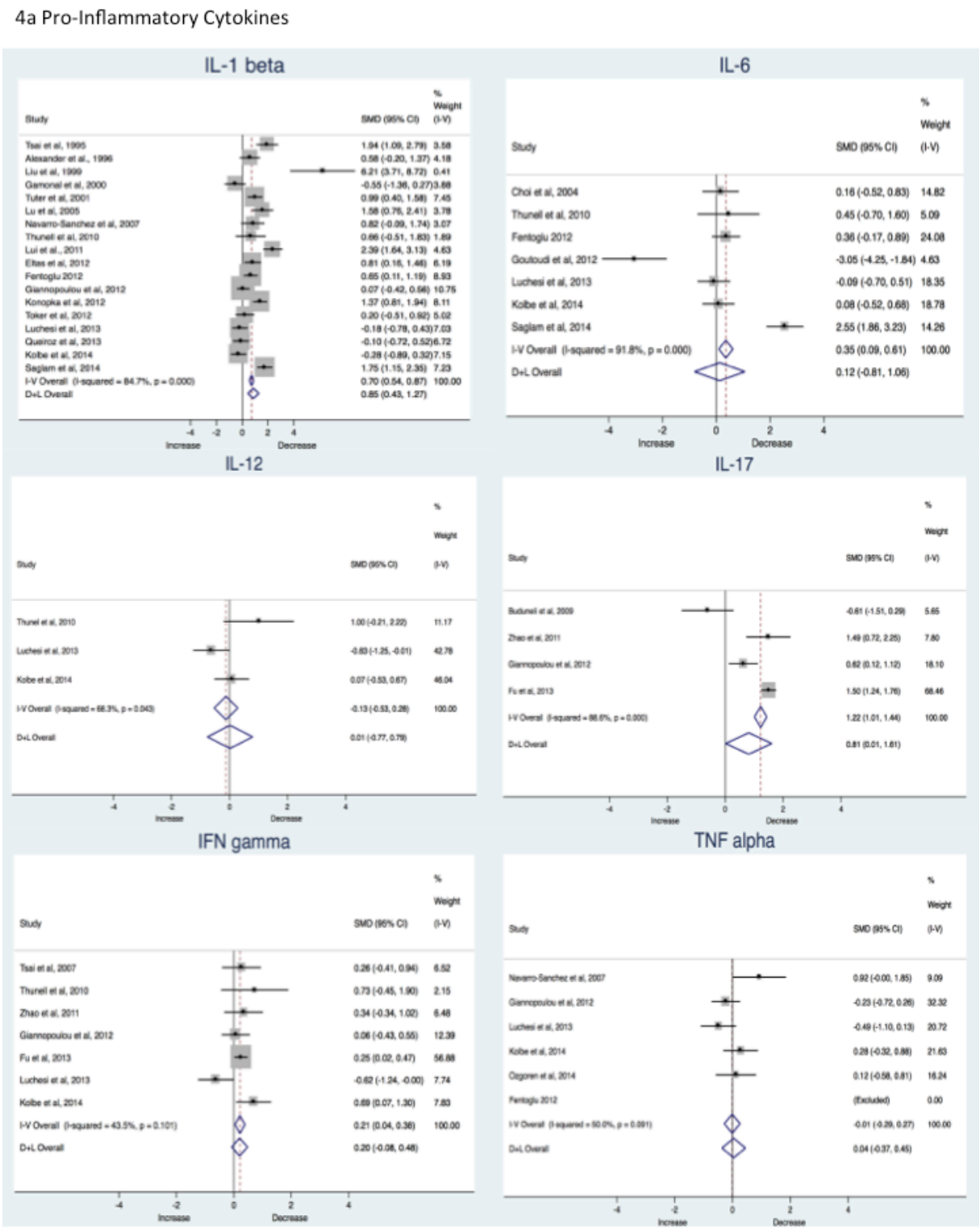


3b. Anti-Inflammatory Cytokines

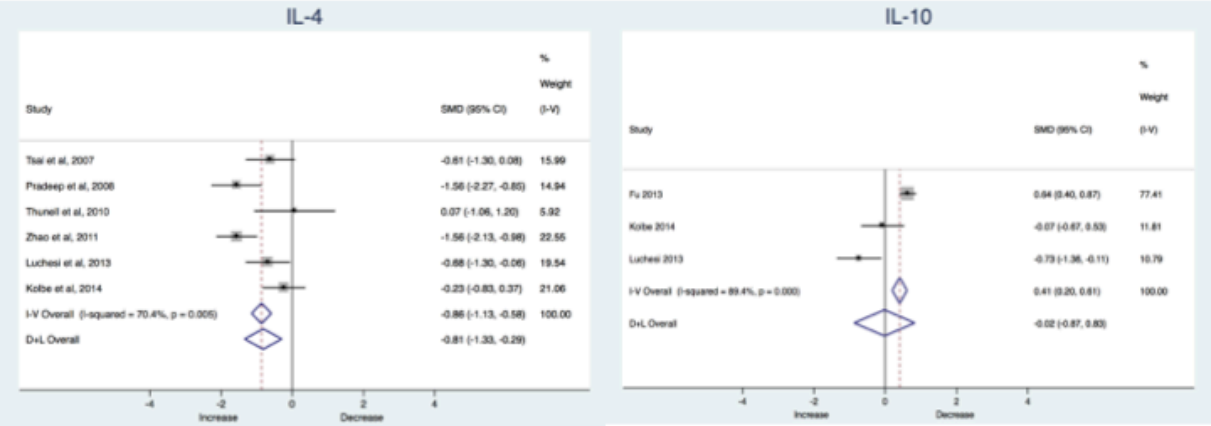


3c. Chemokines

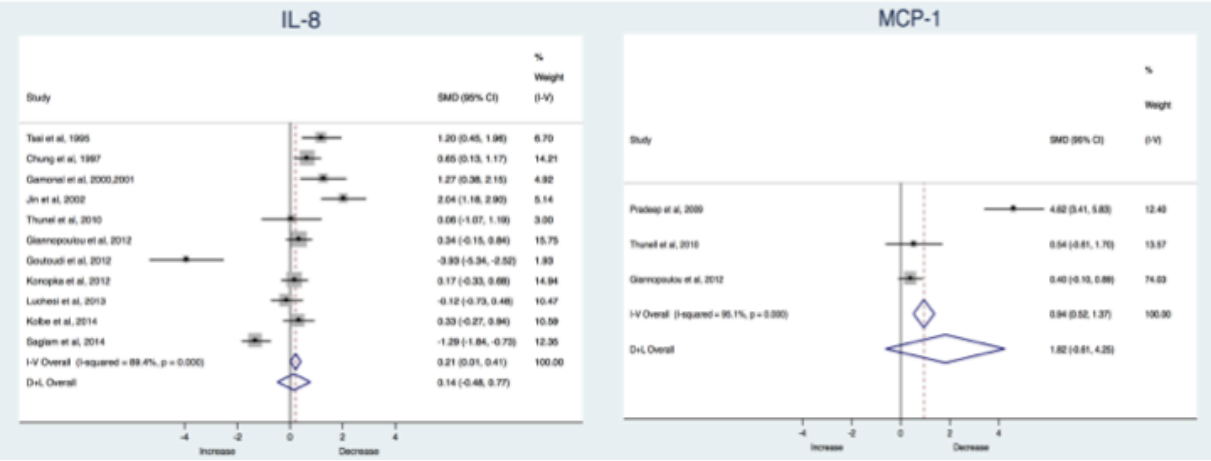
Figure 4. Supplementary file 2.



4b. Anti-Inflammatory Cytokines



4c. Chemokines



3.3 ARTIGO 2: artigo formatado para submissão no Journal of Clinical Periodontology

Effect of supra/subgingival biofilm control during periodontal maintenance on inflammatory markers: a randomized clinical trial

GCF biomarkers during periodontal maintenance

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Key Words: periodontal diseases, long-term care, cytokines, chemokines, dental scaling.

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Clinical Relevance

Scientific rationale for study: The need for patients to be enrolled into periodontal maintenance protocols (PMP) after treatment is well established. However, it is still unknown which interventions are necessary to maintain periodontal health. One possible approach is to determine the impact of different procedures such as supra and supra/subgingival biofilm control performed by the patient and professional.

Principal findings: No significant differences in clinical and immunological parameters were observed between groups.

Practical implications: This study shows that adequate supragingival biofilm control is essential to grant an adequate response at the inflammatory level, maintaining periodontal health up to a 1-year.

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Abstract

Aim: To compare the 1-year effect of two periodontal maintenance protocols (PMP) on gingival crevicular fluid (GCF) levels of pro- and anti-inflammatory biomarkers.

Material and Methods: Following non-surgical periodontal treatment, 34 subjects (14M/20F, mean age: 54 years) diagnosed with chronic periodontitis were randomly assigned into one of the following study arms: a) supragingival scaling only; b) supra and subgingival scaling. All subjects received personalized oral hygiene instructions. Subjects were seen at 3 months intervals for periodontal maintenance, clinical data and GCF collection. GCF levels of 16 different cytokines and chemokines were measured using a multiplex immunoassay.

Results: No significant differences between PMPs were observed for any clinical parameters or immunological biomarkers. Median GCF concentration levels for most pro-inflammatory cytokines were generally low/moderate throughout the study period, with the exception of IL-1β. GCF levels of anti-inflammatory cytokines IL-4 and IL-13 were moderate/high. For chemokines, GCF levels for CCL3 and CCL2 were high, and concentration for IL-8 was very high.

Conclusion: Our findings suggest that a PMP based on supragingival biofilm control alone is as effective in maintaining low levels of periodontal inflammation and clinical stability after treatment as a PMP based on combined supra/subgingival biofilm control (ClinicalTrials.gov # NCT01598155).

It is well established in the literature that periodontitis can be treated successfully by nonsurgical and/or surgical therapies (Sanz et al. 2012), and that high levels of oral hygiene and implementation of a periodontal maintenance program (PMP) are necessary for the long-term stability of clinical outcomes (Nyman et al. 1977, Becker et al. 1984a, Becker et al. 1984c). Professional supragingival scaling and subgingival scaling and root planning (SRP) combined with improved personal oral hygiene has been shown to be effective during treatment in periodontal probing depth (PPD) reduction and clinical attachment level (CAL) gain and, therefore, it is considered the standard of care for periodontitis treatment (Ramfjord 1987, Westfelt et al. 1998, Van der Weijden and Timmerman 2002, Suvan 2005, Cobb 2002, Gomes et al. 2014). In contrast, besides the importance of a good plaque control performed by the patient, it is unclear which clinical procedures should be implemented by a professional in a PMP in order to achieve long-term periodontal stability, and most patients receive a combination of supragingival and subgingival interventions including scaling, root planning and polishing at the discretion of the clinician.

It has been shown that supragingival biofilm control on its own can reduce pocket depth and bleeding on probing on a short-term basis (Gomes et al. 2007, Gomes et al. 2008, Hellstrom et al. 1996). These studies also report significant changes in the subgingival pathogenic microbiota. Few studies have dealt with this issue on a long-term basis. A prospective clinical trial (Jenkins et al. 2000) showed no significant differences in PPD, CAL and bleeding on probing (BOP) between subjects receiving supragingival only or supra/subgingival scaling during periodontal maintenance. To the best of our knowledge no other studies have directly investigated this issue (Heasman et al. 2002). These findings provide a new insight to the possible role that supragingival biofilm control may have on the long-term success of periodontal treatment.

The progression of destructive periodontal disease is a result of the interactions between supra and subgingival bacteria and the immune response by the host (Page et al. 1997). The importance of periodontophatic bacteria both in the supra as well as subgingival areas is fundamental for the initiation and development of this process (Marsh et al. 2011). It is accepted that this inflammatory response is involved with the establishment and progression of the destruction associated with periodontitis (Page et al. 1997). Several mechanisms involving pro-inflammatory and anti-inflammatory interactions have been associated with this process. Studies have

shown higher concentration of pro-inflammatory cytokines and chemokines on diseased than healthy periodontal sites including interleukin (IL)-1β, IL-6, IL-8, macrophage chemotactic protein (MCP-1, also known as CCL2) and tumor necrosis factor alpha (TNF-α) (Fujita et al. 2012, Shimada et al. 2013, Keles et al. 2014). Similarly, studies have shown a significant decrease in these inflammatory markers after periodontal treatment (Eltas and Orbak 2012, Gupta et al. 2013, Giannopoulou et al. 2012). A limited number of studies have focused on the role of anti-inflammatory mediators on periodontal disease. Conflicting results are available regarding the GCF levels of IL-4 and IL-10 in periodontal health and disease (Cetinkaya et al. 2013, Shimada et al. 2013, Thunell et al. 2010, Pradeep et al. 2008). With regards to periodontal treatment, studies have shown that GCF levels of IL-4 increase after periodontal treatment (Luchesi et al. 2013, Zhao et al. 2011, Pradeep et al. 2008), whereas GCF levels of IL-10 remain unchanged (Kolbe et al. 2014).

Therefore, our hypothesis was that a PMP based on a professional supragingival biofilm control only is as effective in achieving low levels of periodontal inflammation after treatment as a PMP based on combined supra/subgingival biofilm control. Confirmation of this hypothesis would have meaningful clinical repercussions since subgingival interventions require local anesthesia, specialized instruments, trained clinicians, longer operatory time and may cause trauma to the periodontal tissues. In this sense, the aim of the present study was to compare the 1-year effect of two PMPs on gingival crevicular fluid (GCF) levels of pro- and anti-inflammatory biomarkers.

Material and Methods

The Ethics Committee of the Federal University of Rio Grande do Sul (UFRGS), Brazil, reviewed and approved the research protocol. All subjects signed an informed consent prior to their enrollment in the study. The study was registered on ClinicalTrials.gov under the identifier NCT01598155.

Study design and sample

This study was a masked, parallel-design, randomized clinical trial. Potential participants were recruited from the dental clinic at the Faculty of Dentistry, UFRGS,

Brazil, between May 2012 and September 2013. Figure 1 depicts the flowchart and study design. The study was conducted in two separate phases — active periodontal treatment and PMP. In brief, 72 out of 118 subjects were initially treated, and then 62 subjects were included in the PMP phase. Ten subjects were discontinued due to low compliance. Herein, we describe the results of a subsample comprised of 34 subjects who were followed-up for 12 months.

Consenting subjects were included if they were 35 years or older, had ≥12 teeth present, and had diagnosis of moderate or advanced chronic periodontitis (Page and Eke 2007, ≥2 interproximal sites with CAL ≥4mm or ≥2 interproximal sites with pocket depth PD ≥5mm). Subjects were not included if they had any systemic condition that could impact periodontal response to treatment, including diabetes, HIV infection, cardiovascular disease, required antibiotic prophylaxis for routine dental procedures, had taken antibiotics in the previous 3 months, received periodontal treatment in the last 12 months, had fixed orthodontics devices or who were pregnant or nursing.

Active periodontal treatment and periodontal maintenance

Following the initial examination, carious lesions and faulty restorations were addressed and remaining roots or hopeless teeth were extracted. Subjects received oral hygiene instructions and supragingival scaling once a week for 4 weeks (Fig. 1). Then, two periodontists (AFS, PDMA) performed quadrant-wise scaling and root planning under local anesthesia i.e., one quadrant per week. Regular toothbrushes, interproximal brushes, dental floss and toothpaste were provided throughout the study as needed. Subjects were asked to avoid mouthrinses.

Subjects were randomized into one of the following study arms for PMP procedures: a) full mouth supragingival scaling only (n=17); and b) full mouth supragingival scaling and subgingival scaling in sites with persistent BOP (n=17). Randomization was performed in blocks of 20 subjects, stratified for smoking status, using a web-based randomization program (www.randomization.com). Allocation concealment was achieved by storing the group assignment into brown envelops, which were handled by an assistant external to the study.

Subjects were seen at 3 months intervals for both experimental groups, and all subjects received personalized oral hygiene instructions and motivation, and tooth polishing throughout the study. PMP procedures were performed by 3 periodontists

not involved with the clinical examinations and biological sampling, with hand instruments.

Exams and clinical procedures

Two calibrated periodontists performed the clinical exams (AFS, PDMA). Periodontal parameters were measured at six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) of all teeth present, excluding third molars. The clinical measurements included visible plaque accumulation (VP, present or absent), gingival bleeding (GB, present or absent), BOP (present or absent), PPD (mm) and CAL (mm). All measurements were performed using a North Carolina probe (Neumar, São Paulo) with millimeter intervals, and the values were rounded up to the nearest millimeter. Intra and inter-examiner calibration were assessed on 7 subjects before the beginning of the study and again on 7 subjects every 12 months. The Intraclass Correlation Coefficients for PPD and CAL were >0.86 for intra-examiner reproducibility and >0.84 for inter-examiner reproducibility. Examiners were masked to the study interventions.

GCF sampling

GCF samples were obtained from 4 sites, which were identified as being the deepest before the active periodontal treatment. These sites were the same throughout the study. The same two blind, calibrated and trained examiners (AFS, PDMA) performed the sampling. After isolation of the area with cotton rolls, supragingival plaque was carefully removed using hand instruments. One paper strip (Periopaper, Oraflow Inc., Plainview, NY, USA) was gently inserted into the gingival crevice, 1-2mm subgingivally, until mild resistance was felt, for 30 seconds. GCF volume was immediately determined using a chair-side instrument (Periotrom 8000, Oraflow Inc., Plainview, NY, USA), calibrated according to the manufacturer's specification. The samples were pooled and immediately placed in Eppendorf tubes containing 50µl of a protease inhibitor cocktail (Complete mini EDTA-free, protease inhibitor cocktail tablets; Roche Applied Science, Indianapolis, IN, USA) and stored at -80°C. Samples visibly contaminated with blood were discarded. GCF sampling was performed at baseline, 3, 6 and 12 months of PMP (Figure 1). At 9 months, clinical exams and procedures were performed, but GCF sampling was not performed, due to the fact that this data would not bring additional information.

Quantification of biomarkers

Cytokine and chemokine concentrations were determined using a multiplex fluorescent bead-based immunoassay system (Bio-Plex MAGPIX Multiplex Reader, Bio-Rad, Hercules, CA, USA), with the proper software (Bio-Plex Manager MP Software, Bio-Rad, Hercules, CA, USA). GCF levels of 16 different cytokines and chemokines were measured using the Cytokine Human Ultrasensitive Magnetic 10-Plex Panel and a custom magnetic cytokine assay (Novex, Multiplex Luminex Assay, Life Technologies, Grand Island, NY, USA). The Ultrasensitive 10-Plex included the pro-inflammatory cytokines IL-1β, IL-2, IL-6, interferon gamma (IFN-γ), TNF-α, and granulocyte macrophage-colony stimulating factor (GM-CSF), the anti-inflammatory cytokines IL-4, IL-5 and IL-10, and the chemokine IL-8. The custom assay included the pro-inflammatory cytokines IL-12(p70p40) and IL-17, the anti-inflammatory cytokine IL-13, and the chemokines CCL2, macrophage inflammatory protein-alpha (MIP-1α, also known as CCL3) and macrophage inflammatory protein-beta (MIP-1β, also known as CCL4).

GCF samples were eluted in 150μl PBS and shaken for 4 hours in an orbital shaker. Aliquots of 50μl of each GCF sample were used to run each assay. The assays were performed in 96-well flat bottom plates following the instructions of the manufacturer. Readings were performed with MAGPIX. The total concentrations of the GCF samples were estimated from the standard curve, which had 9 dilutions, expressed as picograms per milliliter (pg/mL) using the curve fitting software, considering sample dilution. The same protocol was applied for the custom assay. For the Ultrasensitive assay, the limit of the detection was lower than 0.5pg/mL for all analytes. For the custom assay, the limit of detection of each analytes was estimate using the standard curve (functional sensitivity), which included two additional dilutions of the standards (S8 and S9). The lowest limit was 1.19pg/mL for IL-12(p70p40) and the highest was 7.46pg/mL for IL-13. All values below the limit of detection were set to zero.

Statistical Analysis

Clinical data was analyzed using parametric and non-parametric tests. Baseline clinical data was compared between experimental groups using independent t-test,

chi-square test and Mann-Whitney test. Longitudinal clinical data was analyzed using generalized estimating equations and unstructured correlation. Quasi-likelihood under the independence model criterion method was used for model-selection including the correlation structure (Pan 2001).

Cytokine and chemokine data did not follow a normal distribution. Analytes concentrations were presented using box-plots. Crude and adjusted generalized estimating equations were used to estimate treatment effects while accounting for the longitudinal nature of the study. Cytokine and chemokine concentrations were rank-transformed and the quasi-likelihood under the independence model criterion method was used for model-selection including the correlation structure (Pan 2001). An exchangeable correlation structure was used. GCF samples were available for the immunological analysis for all but one participant for the 12 months interval. A last observation carried forward strategy was used for the missing data.

Results

Thirty-four subjects completed the 1-year follow-up, 17 in each experimental group (Figure 1). A total of 136 GCF samples were collected and 135 were analyzed for the presence of 16 cytokines and chemokines.

No significant differences were observed between experimental groups at baseline (Table 1) or overtime (Table 2) for socio-demographic, behavioral and full-mouth clinical variables. When sites used for GCF collection were compared, no significant differences were observed between groups for all clinical parameters but BOP. At baseline, BOP was significantly higher for the supra/subgingival than supragingival only group (27.94 ± 21.44 vs. 51.47 ± 28.60, p=0.02); however, no significant differences were observed between groups after 3 months of follow-up. Importantly, full-mouth VP and GB scores were, respectively, lower than 30% and 10% of sites throughout the study, indicating good supragingival biofilm control. For the 4 sites selected for biological sampling, mean PPD ranged between 2.87±0.59 and 3.50±0.81mm and mean GCF volumes ranged between 1.23±0.43 and 1.52±0.74 μL (Table 2).

Median GCF concentration levels for the pro-inflammatory cytokines were generally low or moderate throughout the experimental period (Figure 2). GCF levels for IFN-γ and GM-CSF were particularly low (<100 pg/mL) approaching the limit of detection of the assays. Conversely, IL-1β had the highest median GCF levels during

the study (>5,000 pg/mL). IL-17, TNF-α, IL-2, IL-12(p70p40) and IL-6 had intermediate levels. Median GCF concentrations of anti-inflammatory cytokines were mixed with IL-4 and IL-13 having high concentration (>2,500 pg/mL) and IL-5 and IL-10 very low concentration (<100 pg/mL) (Figure 3). Chemokines had high median GCF concentration levels with the exception of CCL4 (<200 pg/mL). IL-8 median concentration levels were particularly high (>150,000); this was also true, but to a lesser extent, for CCL2 and CCL3 (>1,500 pg/mL) (Figure 3).

Table 3 presents the effects of PMPs and time on the cytokines and chemokines analyzed. No significant differences were observed between the PMPs for any of the analytes. A significant reduction in the concentration of IL-12(p70p40), IL-4 and IL-13 was observed overtime with no significant differences between PMPs. Adjusting for age, sex, race, socioeconomic status and smoking status in the multivariable analysis did not fundamentally change the results (data not shown).

Discussion

The aim of the present randomized clinical trial was to compare the effect of two PMPs on GCF levels of pro- and anti-inflammatory markers over a 1-year period. GCF levels for most pro-inflammatory cytokines were generally low or moderate, whereas GCF levels of anti-inflammatory cytokines and chemokines were moderate or high; no significant differences were observed between PMPs. Collectively, these findings appears to indicate a PMP based on supragingival biofilm control alone is as effective in achieving resolution of periodontal inflammation and clinical stability after treatment as a PMP based on combined supra/subgingival biofilm control. The clinical data maintained stable during the study period corroborate the findings from pro- and anti-inflammatory markers. To the best of our knowledge this is the first study to investigate the long-term effect of supragingival biofilm control alone on periodontal molecular and clinical parameters during periodontal maintenance.

It is well established that periodontal maintenance is essential for the long-term success of periodontitis treatment. In the classic study by Axelsson et al. (2004), a PMP based on patients needs including personalized oral hygiene instructions and professional mechanical tooth cleaning was very effective in reducing the incidence of caries, attachment loss, and tooth loss over a 30-year follow-up period. This is in accordance to findings from Axelsson and Lindhe (1981) and Becker et al. (1984a), who have indisputably shown disease recurrence in patients periodontally treated but

not maintained. Whereas the need for PMP after periodontal treatment is clear, it is unknown which kinds of interventions are necessary to maintain periodontal health. PMPs have ranged from supragingival scaling/polishing (Westfelt et al. 1998, Lorentz et al. 2009) to subgingival scaling of selected sites and subgingival scaling of all sites (Lindhe and Nyman 1984, Hirschfeld and Wasserman 1978). Irrespective, no preferred protocol can be readily identified from the literature, and a systematic review found that only one study has compared different PMPs directly (Heasman et al. 2002).

Our results support previous clinical findings from Jenkins et al. (2000), who did not find a significant difference in PPD, CAL, and BOP between subjects receiving supragingival or subgingival scaling during PM. Similarities between studies include the study design (randomized clinical trial), the sample size (34 subjects) and follow-up (1-year). Whereas in our study all subjects were treated following a strict nonsurgical treatment protocol by the 2 clinicians and only subjects with good oral hygiene compliance were included in the sample, Jenkins et al. (2000) selected patients who had received nonsurgical and/or surgical periodontal treatment and had remaining deep pockets (4 pockets with PPD >4mm). While the subjects from this study maintained a regular plaque control, subjects from Jenkins study had not consistently achieve an adequate plaque control during their treatment and maintenance phases. Collectively, both studies indicate that supragingival interventions are effective in achieving periodontal stability and minimal local inflammation in periodontally treated subjects, as measured by clinical parameters.

The present study extends the abovementioned clinical findings by assessing a broad array of biomarkers related to the periodontal immuno-inflammatory response. In general, low concentrations of pro-inflammatory and high concentration of anti-inflammatory biomarkers were observed for both groups indicating resolution of the inflammatory process, which corroborates the clinical findings of periodontal stability. Several short-term studies have demonstrated that periodontal treatment reduces significantly pro-inflammatory cytokine and chemokine levels including IL-1β, IL-6, IL-8, IL-12, IL-17, IFN-γ, and CCL2 (Konopka et al. 2012, Thunell et al. 2010, Reis et al. 2014, Fu et al. 2013). Few clinical studies have assessed the impact of periodontal treatment on GCF inflammatory markers for 6 months or more. Eltas and Orbak (2012) showed that GCF levels of IL-1β decreased significantly after treatment and remained low for 9 months. In a 6-months follow-up study, Giannopoulou et al.

(2012) found that GCF levels of IL-17, IFN-γ, TNF-α, GM-CSF, CCL3 decreased significantly after treatment and remained at low levels. Recently, Saglam et al. (2014) showed that the GCF levels of IL-1β and IL-6 decreased significantly after treatment and remained low for up to 6 months. Even though these studies, in general, corroborate our findings, direct comparisons are unwarranted since no description of the post-treatment care was provided in these publications. Overall, the low concentration of pro-inflammatory cytokines and chemokines support our clinical findings, and indicate that both PMPs were able to achieve and maintain minimal periodontal inflammation over a period of 12 months.

Cytokines are secreted by a large range of cells, including T cells, in response to antigens that have been recognized by antigen-presenting cells (Cekici et al. 2014). Activated naïve T cells mature into different subsets of T helper cells (T_h) including T_{h1}, T_{h2}, and T_{h17} and in T regulatory cells (T_{reg}), which play an important role in periodontal disease (Gaffen and Hajishengallis 2008). In general, T_{h1} and T_{h17} secrete mainly pro-inflammatory cytokines, and T_{h2} and T_{reg} secrete mainly anti-inflammatory cytokines. Periodontal health has been characterized by low levels of pro-inflammatory cytokines and high levels of cytokines that suppress the immune-inflammatory response (anti-inflammatory cytokines), and the opposite has been observed in periodontitis (Page et al. 1997, Cekici et al. 2014). In the present study, periodontally treated subjects were maintained following two PMPs, thus we expected to observed low values for pro-inflammatory cytokines similar to what has been observed in periodontally health subjects. With the exception of IL-1β, all other pro-inflammatory cytokines had moderate to low GCF concentration values. Explanation for the observed high GCF levels of IL-1 β is that subjects with previous history of periodontitis may have inherently higher IL-1β GCF levels. (Figueredo et al., 1999) showed that high levels of IL-1β are typical of patients with periodontitis, regardless of the severity of disease at the sampled site. Noteworthy to mention that the high degree of heterogeneity observed in the literature including inconsistent inclusion criteria, disease definition, control of important co-factors (smoking), GCF sampling methodology, reporting, treatment protocols and follow-up intervals complicates any direct comparisons with our findings.

Among the four anti-inflammatory cytokines studied, subjects had high values of IL-4 and IL-13, and low levels for IL-5 and IL-10. Studies support our findings with regards to IL-4 (Pradeep et al. 2008, Luchesi et al. 2013, Zhao et al. 2011, Cetinkaya

et al. 2013), whereas limited data about IL-13 is available (Luchesi et al. 2013, Kolbe et al. 2014). It has been shown that IL-10 is highly expressed in inflamed periodontal tissues, where it is thought to limit disease severity (Lappin et al. 2001, Garlet et al. 2004, Garlet et al. 2006). The limited amount of periodontal inflammation observed during PMP for both groups may explain the low levels of IL-10 observed. Limited information is available about the role, if any, of IL-5 in periodontal disease. Nevertheless, results from an animal study suggest that the regulation of this cytokine can be affected by oral pathogens, such as *Porphyromonas gingivalis* (Card et al. 2010).

Chemokines are chemotactic cytokines that bind to specific receptors and selectively attract different cell subsets to the inflammatory site (Garlet et al. 2003). High values of IL-8, CCL2 and CCL3 were observed throughout the experimental period. The high levels of IL-8 throughout the study might be explained by its role in cell proliferation and angiogenesis during wound healing (Takigawa et al. 1994, Zlotnik et al. 1999), and by its high expression in epithelial cells even in non-inflamed gingiva. IL-8 plays an important role in the establishment of equilibrium between the continuous bacterial challenge and the host defense (Sfakianakis et al. 2002). CCL2 and CCL3 are chemotactic for monocytes or lymphocytes and determine the transition of the acute into chronic inflammatory process. Those chemokines also regulate bone remodeling through osteoclast activity (Haytural et al. 2015, Jiang and Graves 1999, Yu et al. 1993). The significance of the elevated levels of CCL2 and CCL3 in this study is currently unknown.

Clinical studies have shown a positive effect of supragingival biofilm control on the composition of subgingival biofilm and periodontal parameters. Gomes et al. (2007) and (2008) showed that supragingival scaling followed by strict supragingival biofilm control significantly improved the clinical parameters and subgingival total bacteria counts biofilm for up to 6 months. Ximenez-Fyvie et al. (2000) showed a significant decrease in subgingival periodontal pathogens in subjects who received professional supragingival plaque removal after SRP. Collectively, these findings support the hypothesis that supragingival biofilm control decreases subgingival biofilm species, which leads to decreased periodontal inflammation among patients with history of periodontitis. Interestingly, the well-established routine scale and polish of periodontally healthy subjects has been questioned in a recent systematic review (Worthington et al. 2013).

Among the limitations of the present publication are the limited sample size and follow-up time due to the ongoing nature of the study. The 4 sites selected for GCF collection were identified at the initial visit, and this strategy may have resulted in the selection of sites, which were not the deepest sites after periodontal treatment. Nevertheless, this strategy is likely to similarly influence both groups. At baseline, experimental groups were comparable in all but one parameter; frequency of BOP in sites selected for GCF analyses being significantly higher in the supra/subgingival than supragingival only group. This significant difference between groups was not observed in any of the other experimental periods. Additionally, this significant difference was not corroborated by differences in GCF volume or cytokine/chemokine levels at baseline. Exploratory analysis taking into consideration the whole sample did not yield significant differences between experimental groups for BOP at baseline or overtime. The strengths of the study include the study design, standardization of procedures, good overall results of the nonsurgical periodontal treatment, and wide array of immuno-inflammatory biomarkers.

In conclusion, the results suggest that subgingival scaling during PMP does not provide additional clinical and immunological benefits to periodontally treated compliers patients when compared to supragingival scaling alone.

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Figure Legends

Figure 1. Flow-chart and study design.

Figure 2. Pro-inflammatory cytokines distribution between groups overtime. spg – supragingival biofilm control; spg+sbg – supra/subgingival biofilm control.

Figure 3. Anti-inflammatory cytokines and chemokines distribution between groups overtime. spg – supragingival biofilm control; spg+sbg – supra/subgingival biofilm control.

Tables

Table 1. Sample characteristics at baseline

	spg (n=17)	spg+sbg (n=17)	p-value
Demographics, socioeconomic and behavioral variables			
Age (mean±SD)*	53.70±9.75	53.35±8.87	0.91
Sex (M/F)**	7/10	7/10	1.00
Skin color (white/non-white)**	6/11	5/12	0.71
Socioeconomic status (high/low)**	4/13	6/11	0.45
Smoking status (smokers/non-smokers)**	4/13	3/14	0.67
Full-mouth clinical parameters			
Visible plaque (%)***	29.54±14.47	29.45±11.90	0.80
Gingival bleeding (%)***	9.31±8.47	9.47±9.86	0.89
BOP (%)***	19.43±9.47	19.77±7.57	0.88
PPD (mm)*	2.32±0.37	2.13±0.34	0.11
CAL (mm)*	3.35±0.99	3.20±0.72	0.61
Clinical parameters of sites selected for GCF sampling (mean±SD)			
Visible plaque (%)***	45.59±30.92	38.23±25.18	0.51
Gingival bleeding (%)***	10.29±15.46	10.29±17.81	0.83
BOP (%)***	27.94±21.44	51.47±28.60	0.02
PPD (mm)*	3.50±0.81	3.25±0.69	0.34
CAL (mm)*	4.50±0.91	4.38±0.87	0.70
GCF (µl)*	1.40±0.80	1.39±0.74	0.96

spg – supragingival biofilm control; spg+sbg – supra/subgingival biofilm control; *Independent t-test; **chi-square test; ***Mann-Whitney test.

Table 2. Clinical parameters according to experimental groups and time.

	Baseline		3 months		6 months		12 months		
	spg	spg+sbg	spg	spg+sbg	spg	spg+sbg	spg	spg+sbg	p-value*
Clinical parameters on full mouth									
VP	29.54±14.46	29.45±11.91	31.23±17.56	26.19±18.70	28.22±22.27	27.47±18.45	23.32±16.04	26.96±13.85	0.99
GB	9.31±8.46	9.47±9.86	8.42±6.21	7.68±8.75	7.09±10.15	7.78±6.64	2.85±2.92	3.35±3.35	0.97
BOP (%)	19.43±9.47	19.77±7.57	17.30±9.33	17.64±9.32	17.03±8.29	17.70±11.25	13.24±8.03	16.32±8.51	0.64
PPD (mm)	2.32±0.37	2.12±0.34	2.27±0.35	2.12±0.35	2.26±0.34	2.07±0.30	2.24±0.36	2.14±0.37	0.15
CAL (mm)	3.35±0.99	3.20±0.72	3.29±0.88	3.19±0.73	3.33±0.92	3.16±0.71	3.32±0.89	3.22±1.64	0.64
Clinical parameters on sampled sites									
VP	45.59±30.92	38.24±25.18	44.12±30.01	35.29±30.69	38.24±35.49	39.71±31.94	33.82±27.87	48.53±29.94	0.82
GB	10.29±15.46	10.29±17.81	11.76±15.61	14.71±21.76	11.76±19.99	11.76±23.58	1.47±6.06	2.94±8.30	0.76
BOP (%)	27.94±21.44	51.47±28.60	23.53±16.47	32.35±22.99	20.58±15.89	22.06±24.82	20.59±22.07	25.00±21.65	0.02
PPD (mm)	3.50±0.81	3.25±0.69	3.09±0.77	2.93±0.79	3.18±0.90	2.87±0.59	3.21±0.85	2.96±0.79	0.28
CAL (mm)	4.50±0.91	4.38±0.87	4.35±0.99	4.03±0.88	4.26±1.03	4.07±1.02	4.51±1.12	4.09±0.94	0.36
GCF (µl)	1.41±0.80	1.39±0.74	1.45±0.85	1.29±0.51	1.43±0.68	1.23±0.43	1.52±0.74	1.43±0.69	0.52

spg – supragingival biofilm control; spg+sbg – supra/subgingival biofilm control; VP – visible plaque; GB – gingival bleeding; BOP – bleeding on probing; PPD – periodontal probing depth; CAL – clinical attachment loss; GCF – gingival crevicular fluid; * p-value for comparison between experimental groups adjusted for follow-up using generalized estimating equations.

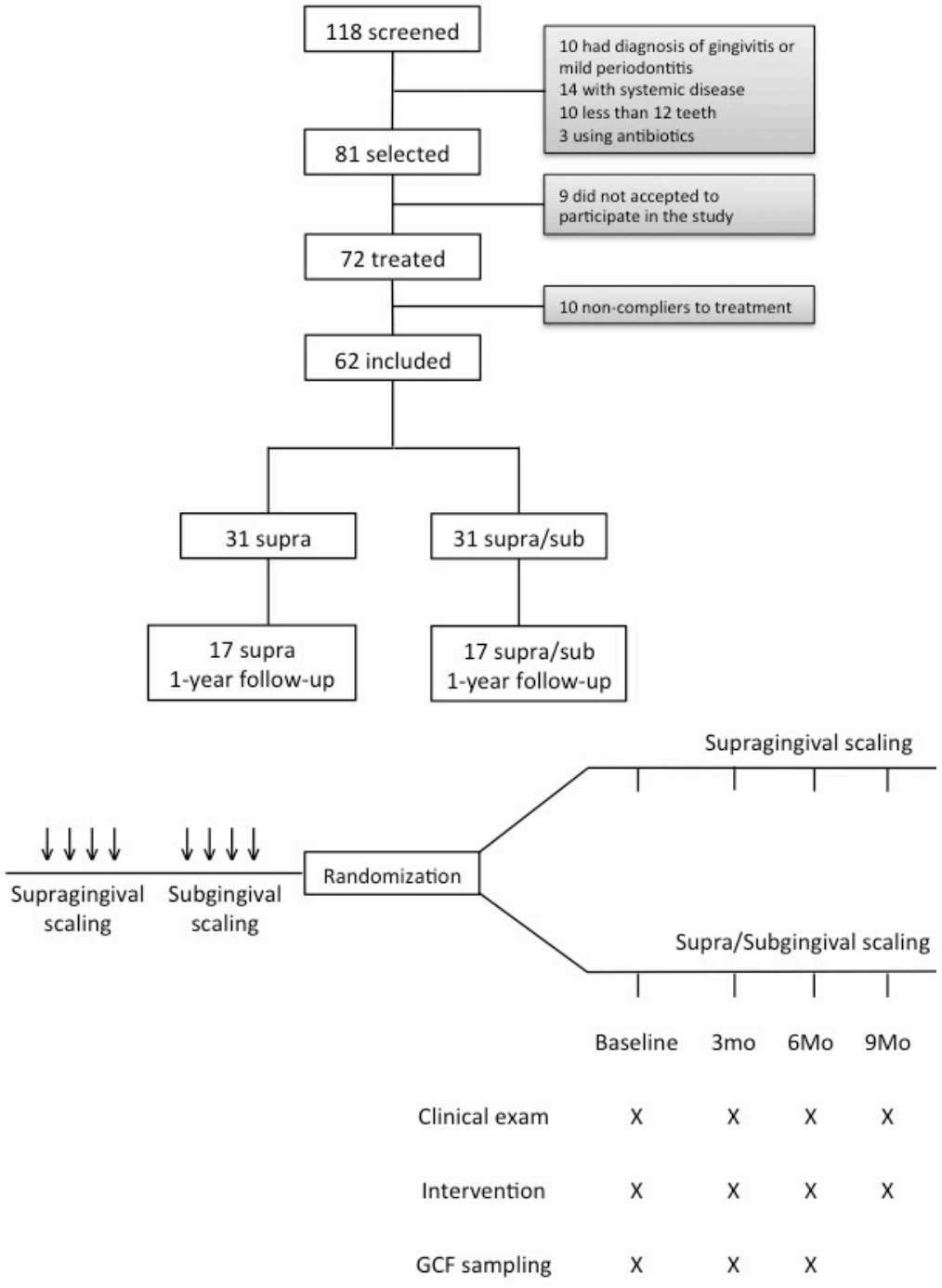
Table 3. Generalized estimating equations for the effect of PMPs and time on GCF concentration of cytokines and chemokines (n=34).

Pro-inflammatory cytokines								
	IL-1β		IL-2		IL-6		IL-12(p40p70)	
	Coef	p-value	Coef	p-value	Coef	p-value	Coef	p-value
spg	Ref		Ref		Ref		Ref	
spg+sbg	0.26 ± 10.99	0.981	2.56 ± 11.13	0.818	6.35 ± 11.25	0.573	-9.06 ± 9.11	0.320
Baseline	Ref		Ref		Ref		Ref	
3 months	-2.47 ± 6.34	0.697	1.03 ± 6.15	0.867	4.55 ± 5.91	0.440	-10.01 ± 7.07	0.157
6 months	-2.57 ± 6.34	0.685	0.81 ± 6.15	0.895	-3.35 ± 5.91	0.570	-20.34 ± 7.07	0.004
12 months	-4.48 ± 6.34	0.479	-3.48 ± 6.14	0.571	2.55 ± 5.91	0.665	-16.12 ± 7.07	0.023
	IL-17		IFN-γ		TNF-α		GM-CSF	
spg	Ref		Ref		Ref		Ref	
spg+sbg	13.46 ± 12.14	0.268	3.12 ± 11.21	0.781	0.82 ± 11.06	0.941	6.09 ± 12.21	0.618
Baseline	Ref		Ref		Ref		Ref	
3 months	-0.66 ± 3.32	0.842	0.09 ± 6.01	0.988	-0.97 ± 6.26	0.877	5.59 ± 4.48	0.212
6 months	-1.81 ± 3.32	0.586	-7.23 ± 6.01	0.225	-0.75 ± 6.26	0.905	-1.39 ± 4.48	0.755
12 months	-5.38 ± 3.35	0.109	-2.79 ± 6.01	0.642	-4.39 ± 6.25	0.482	-2.31 ± 4.48	0.606
Anti-inflammatory cytokines								
	IL-4		IL-5		IL-10		IL-13	
spg	Ref		Ref		Ref		Ref	
spg+sbg	1 ± 11.51	0.931	7.41 ± 12.32	0.547	7.85 ± 12.44	0.528	7.69 ± 12.02	0.522
Baseline	Ref		Ref		Ref		Ref	
3 months	-7.24 ± 5.48	0.187	-2.62 ± 4.25	0.538	3.16 ± 4.02	0.431	-12.26 ± 3.97	0.002
6 months	-13.45 ± 5.48	0.014	-6.76 ± 4.25	0.112	-1.63 ± 4.02	0.685	-15.00 ± 3.97	0.001
12 months	-14.13 ± 5.48	0.010	-5.97 ± 4.25	0.160	-0.12 ± 4.02	0.977	-27.62 ± 3.97	0.001
Chemokines								
	IL-8		CCL2		CCL3		CCL4	
spg	Ref		Ref		Ref		Ref	
spg+sbg	8.94 ± 9.38	0.341	10.09 ± 11.57	0.383	12.46 ± 12.18	0.307	-4.47 ± 9.76	0.647
Baseline	Ref		Ref		Ref		Ref	
3 months	0.25 ± 7.74	0.974	-1.97 ± 5.36	0.713	-2.50 ± 4.24	0.556	0.74 ± 6.68	0.912
6 months	1.36 ± 7.74	0.860	3.10 ± 5.36	0.562	0.19 ± 4.24	0.964	1.72 ± 6.68	0.797
12 months	-5.14 ± 7.74	0.506	-6.66 ± 5.36	0.214	-3.81 ± 4.24	0.369	-4.10 ± 6.68	0.539

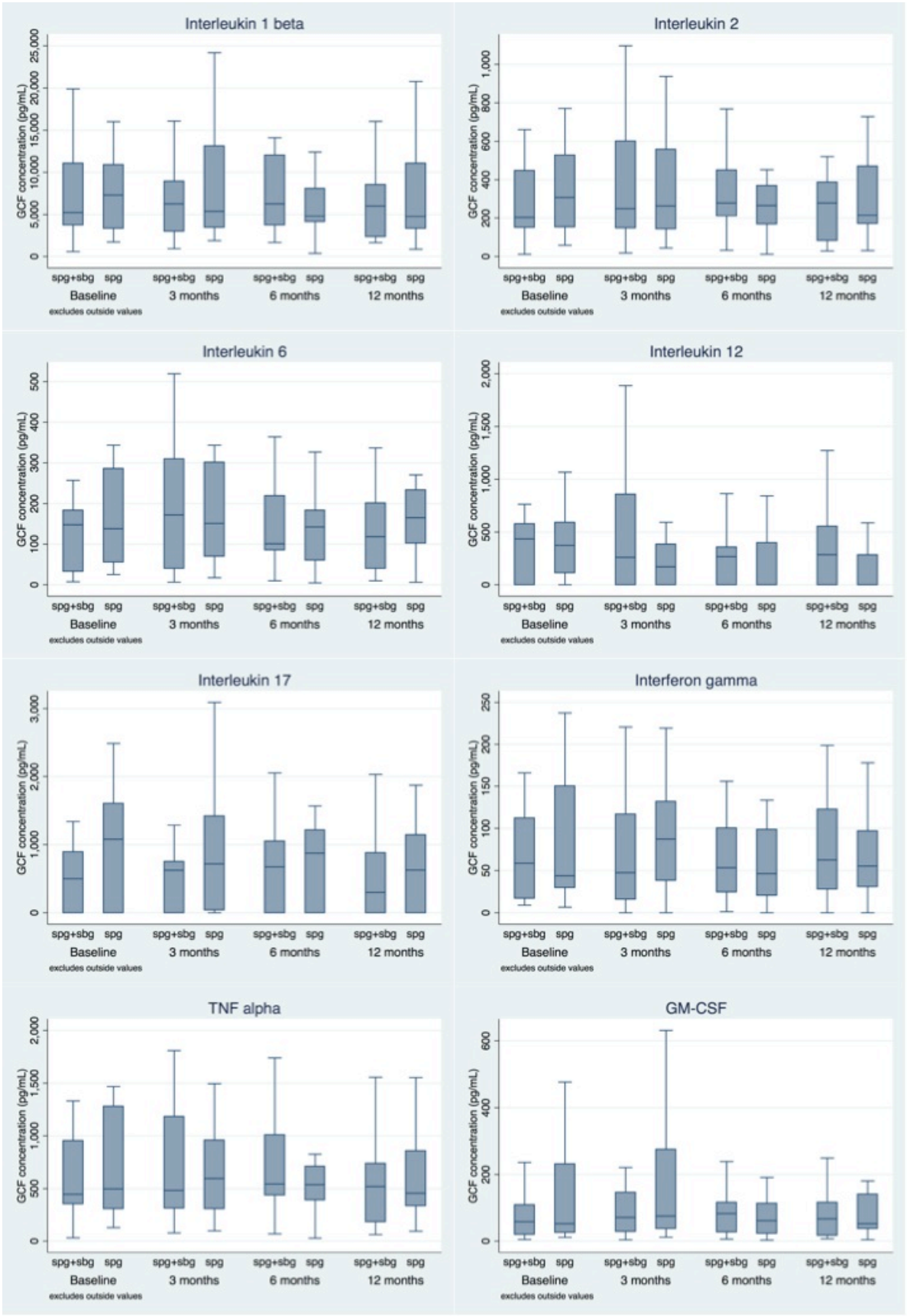
spg – supragingival biofilm control; spg+sbg – supra/subgingival biofilm control; coef – coefficient; ref – reference.

3.4 Figuras do artigo 2

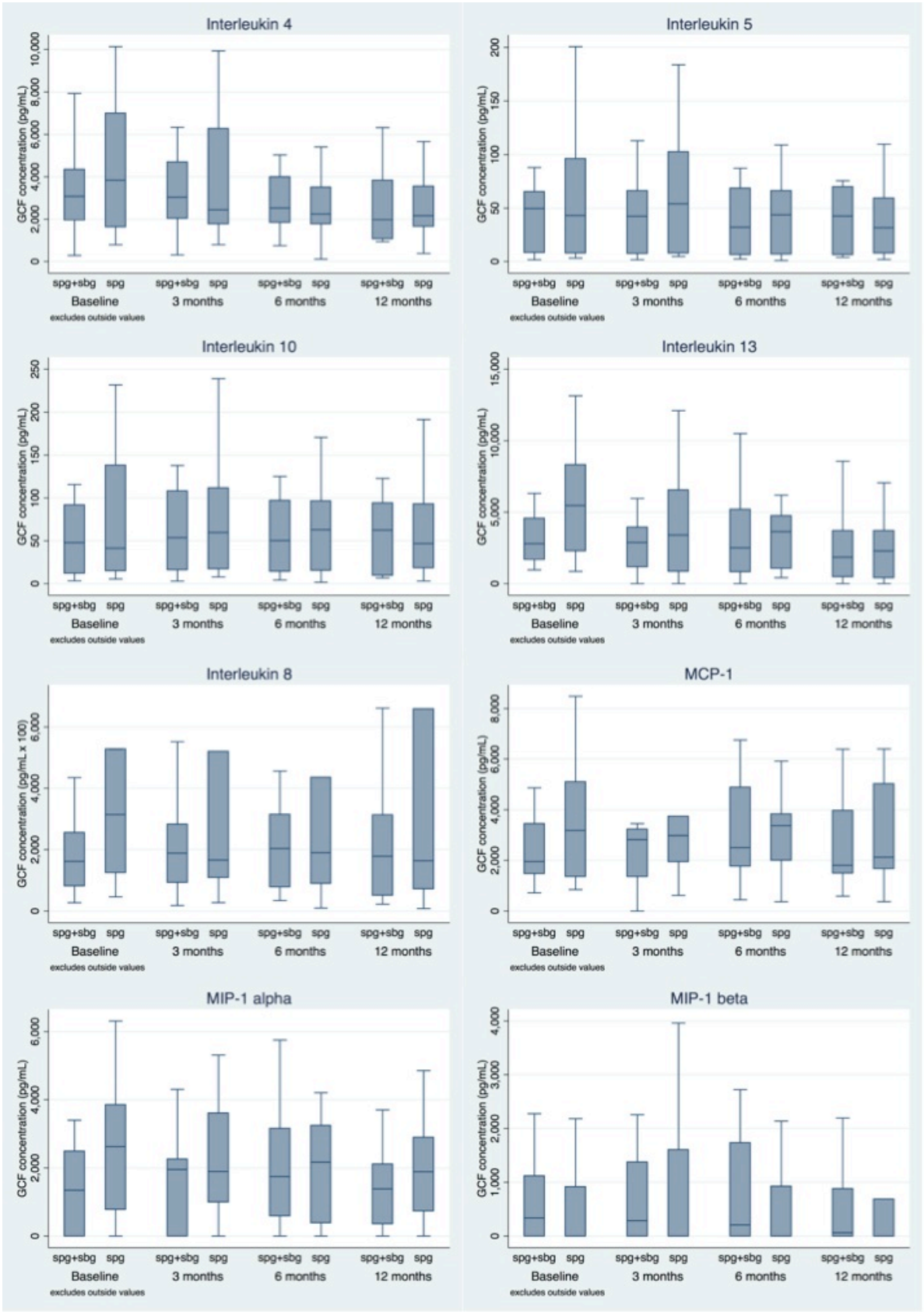
3.4.1 Figure 1.



3.4.2 Figure 2.


Interleukin 1 beta
GCF concentration (pg/mL)
0
5,000
10,000
15,000
20,000
25,000
spg+sbg spg
Baseline
3 months
6 months
12 months
excludes outside values
Interleukin 2
GCF concentration (pg/mL)
0
200
400
600
800
1,000
Interleukin 6
GCF concentration (pg/mL)
0
100
200
300
400
500
Interleukin 12
GCF concentration (pg/mL)
0
500
1,000
1,500
2,000
Interleukin 17
GCF concentration (pg/mL)
0
1,000
2,000
3,000
Interferon gamma
GCF concentration (pg/mL)
0
50
100
150
200
250
TNF alpha
GCF concentration (pg/mL)
0
500
1,000
1,500
2,000
GM-CSF
GCF concentration (pg/mL)
0
200
400
600

3.4.3 Figure 3


Interleukin 4
GCF concentration (pg/mL)
0
2,000
4,000
6,000
8,000
10,000
spg+sbg spg
Baseline
3 months
6 months
12 months
excludes outside values
Interleukin 5
GCF concentration (pg/mL)
0
50
100
150
200
Interleukin 10
GCF concentration (pg/mL)
0
50
100
150
200
250
Interleukin 13
GCF concentration (pg/mL)
0
5,000
10,000
15,000
Interleukin 8
GCF concentration (pg/mL x 100)
0
2,000
4,000
6,000
MCP-1
GCF concentration (pg/mL)
0
2,000
4,000
6,000
8,000
MIP-1 alpha
GCF concentration (pg/mL)
0
2,000
4,000
6,000
MIP-1 beta
GCF concentration (pg/mL)
0
1,000
2,000
3,000
4,000

4 CONSIDERAÇÕES FINAIS

Os resultados desta tese sugerem que um procedimento de manutenção periodontal baseado apenas no controle supragengival do biofilme, realizado pelo binômio paciente-profissional, é tão efetivo em manter baixos níveis de inflamação periodontal e estabilidade dos parâmetros clínicos após o tratamento periodontal, quanto uma manutenção baseada no controle do biofilme supra e subgengival associados, por um período de 1 ano.

Os dados imunológicos aqui apresentados vem ao encontro dos resultados clínicos anteriormente apresentados por Jenkins et al. (2000)[18], no qual os autores não encontraram diferenças significativas na profundidade de sondagem, perda de inserção e sangramento a sondagem entre pacientes recebendo apenas controle supragengival e pacientes recebendo controle supra e subgengival do biofilme. Ambos os estudos tiveram duração de 12 meses com visitas de manutenção a cada 3 meses, no entanto, enquanto o presente estudo foi realizado em pacientes tratados seguindo um protocolo rigoroso não-cirúrgico, e a média de presença de placa visível foi mantida abaixo de 35% durante toda a realização do experimento, o estudo de Jenkins et al. (2000)[18] selecionou pacientes que receberam diferentes formas de tratamento (cirúrgico e não-cirúrgico) e o índice de placa foi medido apenas ao final do experimento, com resultados acima de 53%. Além disso, esta tese fornece resultados adicionais ao mostrar que os níveis de biomarcadores inflamatórios tais como citocinas pró- e anti-inflamatórias e quimiocinas relacionadas com a doença periodontal e presentes no fluido crevicular gengival não apresentaram diferenças entre os grupos.

Entre os pontos fortes desta tese estão o modelo (ensaio clínico randomizado), a metodologia bem delineada, com critérios de inclusão e exclusão bem definidos, a periodicidade e colaboração dos pacientes, os resultados satisfatórios do tratamento periodontal realizado (apêndice 6.6), com a manutenção dos resultados clínicos ao longo do tempo e o número de biomarcadores analisados. No entanto, este estudo também apresenta algumas limitações. As análises foram realizadas com uma subamostra de um ensaio clínico randomizado, composta pelos 34 primeiros sujeitos que completaram 12 meses de acompanhamento. Os resultados aqui apresentados devem ser corroborados após o término do estudo, composto por 62 sujeitos. No entanto, análises exploratórias com o número total de

sujeitos incluídos mostraram que não houveram diferenças significativas nos parâmetros clínicos periodontais e microbiológicos entre os grupos (dados não apresentados). Uma vez que os parâmetros inflamatórios clínicos, principalmente sangramento à sondagem, não apresentaram diferenças, espera-se que também não existam diferenças nos parâmetros inflamatórios imunológicos. Além disso, a seleção de sítios para coleta de dados foi realizada antes do tratamento, e, desta forma, é possível que os sítios incluídos no estudos não fossem os sítios mais profundos presentes após o tratamento. No entanto, por se tratar de um estudo randomizado, espera-se que esta distribuição tenha ocorrido igualmente entre os grupos, não acarretando em prejuízo aos resultados.

Intervenções subgengivais requerem anestesia local, utilização de instrumentos específicos, clínicos ou periodontistas bem treinados, maior tempo clínico dedicado, além de poder causar trauma aos tecidos periodontais. Desta forma, os resultados do presente estudo mostram que uma intervenção menos invasiva, com menor risco de lesão aos tecidos é também eficaz em manter níveis clínicos e inflamatórios compatíveis com saúde periodontal.

Por fim, os resultados apresentados permitem concluir que a realização do controle do biofilme supragengival como única forma de intervenção após o tratamento periodontal não-cirúrgico, tem o mesmo efeito do controle do biofilme supragengival acrescido do controle profissional do biofilme subgengival sobre os indicadores imunológicos periodontais presentes no fluido crevicular gengival de pacientes periodontais tratados, ao longo de 12 meses de manutenção periódica preventiva.

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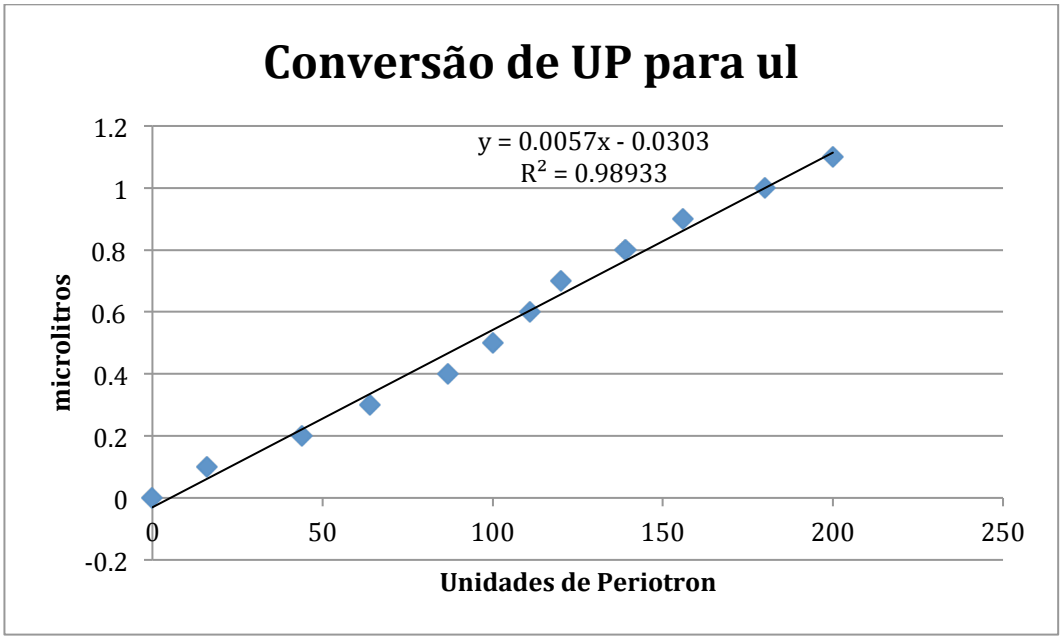
6 APÊNDICE

6.1 Curva-padrão constituída de conversão de Unidades de Periotron para microlitros.



·es de mediana total de todas as citocinas incluídas no Artigo 2.

matory Ctokines	IL-1β	IL-2	IL-6	IL12(p70p40)	IL-17	IFN-γ	TNF-α	GM-CSF
	5715.08	262.32	138.76	245.91	592.90	55.61	523.90	63.96
matory cytokines and chemokines	IL-4	IL-5	IL-10	IL-13	IL-8	CCL2	CCL3	CCL4
	2547.72	42.82	51.94	2753.69	178652	2528.61	1793.18	186.38

·es de mediana e intervalos intequartis para as citocinas pró-inflamatórias incluídas no Artigo 2 ao longo do te

	Baseline Median [25%-75%]	3 months Median [25%-75%]	6 months Median [25%-75%]	12 months Median [25%-75%]	p·
ıpra	7308.79 [3318.6–10886.68]	5380.64 [3399.96–13135.73]	4806.77 [4084.96–8055.7]	4769.47 [3315.89–11083.41]	
ıpra/ Sub	5236.07 [3691.55–11060.93]	6262.24 [2960.75–8952.01]	6268.36 [3711.43–12043.28]	6006.03 [2351.52–8502.9]	
/alue	NS	NS	NS	NS	
ıpra	308.06 [151.78-527.37]	263.79 [143.23-559.26]	266.14 [167.02-369.05]	214.89 [169.11-469.48]	
ıpra/Sub	202.62 [151.02-446.52]	250.01 [148.06-600.23]	279.2 [211.54-449.87]	279.2 [81.4-386.2]	
/alue	NS	NS	NS	NS	
ıpra	138.05 [55.86-285.37]	151.01 [69.81-301.74]	142.4 [59.99-183.01]	165.09 [101.62-233.08]	
ıpra/ Sub	147.82 [33.23-182.71]	172.18 [39.59-309.9]	101.04 [85.74-219.54]	118.39 [40.47-200.83]	
/alue	NS	NS	NS	NS	
ıpra	374.04 [110.64-590.72]	171.11 [0.01-383.26]	0.01 [0.01-399.83]	0.01 [0.01-283.72]	
ıpra/ Sub	434.7 [0.01-577.82]	260.33 [0.01-856.39]	267.27 [0.01-358.4]	285.37 [0.01-553.63]	
/alue	NS	NS	NS	NS	
ıpra	1078.22 [0.01-1604.31]	717.67 [40.09-1,421.13]	873.83 [0.01-1,211.81]	626.71 [0.01-1,139.21]	
ıpra/ Sub	500.56 [0.01-889.36]	624.26 [0.01-753.72]	673.52 [0.01-1,047.98]	494.09 [0.01-773.86]	
/alue	NS	NS	NS	NS	
ıpra	43.79 [29.45-149.93]	87.31 [38.21-131.85]	46.4 [20.25-98.55]	55.35 [30.94-96.62]	
ıpra/ Sub	58.7 [16.71-112.45]	47.52 [15.83-116.9]	53.39 [24.38-100.21]	62.64 [27.9-122.45]	
/alue	NS	NS	NS	NS	
ıpra	498.25 [308.08-1281.54]	596.05 [303.66-956.28]	537.77 [387.45-710.87]	453.14 [331.69-855.3]	
ıpra/ Sub	443.17 [349.63-952.99]	483.59 [311.1-1183.6]	543.92 [436.07-1007.87]	519 [183.9-738.84]	
/alue	NS	NS	NS	NS	
ıpra	52.08 [25.66-231.78]	74.79 [37.22-275]	61.25 [23.13-112.63]	52 [36.41-139.97]	
ıpra/ Sub	57.92 [20.28-108.15]	70.72 [28.45-145.67]	82.3 [26.55-115.45]	66.53 [16.53-115.09]	
/alue	NS	NS	NS	NS	

ıey test was used for comparisons between experimental groups; Kruskal-Wallis test followed by Dunn test were used for comparisons between ti

res de mediana e intervalos intequartis para as citocinas anti-inflamatórias incluídas no Artigo 2 ao longo do te

	Baseline Median [25%-75%]	3 months Median [25%-75%]	6 months Median [25%-75%]	12 months Median [25%-75%]	p-va
upra	3838.19 [1608.81-6994.22]	2445.99 [1763.18-6277.18]	2246.73 [1762.61-3496.12]	2169.84 [1634-3556.59]	NS
upra/ Sub	3078 [1947.47-4346.28]	3039.88 [2039.16-4691.21]	2528.83 [1818.12-4003.05]	1976.4 [1065.12-3831.51]	NS
-value	NS	NS	NS	NS	
upra	43.15 [7.41-96.24]	54.03 [7.65-102.57]	43.8 [6.88-66.32]	31.39 [7.82-59.41]	NS
upra/Sub	49.72 [7.88-65.18]	42.44 [6.97-66.05]	31.85 [6.38-68.43]	42.62 [6.2-69.91]	NS
-value	NS	NS	NS	NS	
upra	41.47 [14.94-138.33]	59.75 [17.06-111.59]	62.92 [15.53-96.36]	46.87 [18.12-93.02]	NS
upra/ Sub	47.97 [11.77-91.53]	53.75 [15.81-108.19]	50.35 [13.89-96.66]	62.52 [9.77-94.1]	NS
-value	NS	NS	NS	NS	
upra	5461.41 [2305.32-8328.5]	3406.37 [841.6-6580.33]	3642.97 [1056.55-4723.09]	2298.08 [397.12-3692.06]	NS
upra/ Sub	2803.33 [1675.91-4555.62]	2890.55 [1169.5-3942.87]	2513.75 [816.1-5199.2]	1865.67 [466.34-3716.04]	NS
	NS	NS	NS	NS	

ney test was used for comparisons between experimental groups; Kruskal-Wallis test followed by Dunn test were used for comparisons between ti

res de mediana e intervalos intequartis para as quimiocinas incluídas no Artigo 2 ao longo do tempo.

	Baseline Median [25%-75%]	3 months Median [25%-75%]	6 months Median [25%-75%]	12 months Median [25%-75%]
upra	314102.1 [125361.3-527508.9]	165998.1 [108592.5-520582.6]	190036 [88947.97-435529.8]	163776.4 [70994.68-657755.7]
upra/ Sub	161779.8 [80626.15-255519.3]	188388.9[91803.51-282696.4]	203714.7 [78874.37-315189.3]	178586.9 [51174.96-313261.4]
-value	NS	NS	NS	NS
upra	3178.75 [1350.51-5089.84]	2977.2 [1931.12-3740.38]	3368.75 [1997.73-3824.9]	2125.09 [1662.17-5010.47]
upra/ Sub	1953.4 [1475.68-3436.85]	2817.21 [1350.25-3233.59]	2500.41 [1755.51-4880.05]	1804.65 [1499.18-3966.28]
-value	NS	NS	NS	NS
upra	2623.98 [769.33-3850.69]	1893.67 [999.93-3601.89]	2168.89 [387.73-3241.48]	1887.02 [733.99-2887.34]
upra/ Sub	1345.42 [0.01-2493.61]	1954.06 [0.01-2256.29]	1745.41 [595.07-3150.52]	1385.34 [356.63-2106.25]
-value	NS	NS	NS	NS
upra	0.01 [0.01-914.26]	0.01 [0.01-1,601.39]	0.01 [0.01-924.83]	0.01 [0.01-688.88]
upra/ Sub	338.58 [0.01-1112.63]	286.32 [0.01-1373.12]	207.89 [0.01-1,732.17]	61.73 [0.01-881.05]
-value	NS	NS	NS	NS

ney test was used for comparisons between experimental groups; Kruskal-Wallis test followed by Dunn test were used for comparisons between ti

6.6 Características clínicas da amostra antes e após o tratamento periodontal (Paired T-test)

Full-mouth	Before treatment	After treatment	p-Value
Visible plaque (%)	75.94±17.39	29.50±13.05	<0.001
Gingival bleeding (%)	37.94±20.83	9.40±9.06	<0.001
BOP (%)	94.12±18.52	19.60±8.44	<0.001
PPD (mm)	3.12±0.59	2.23±0.36	<0.001
CAL (mm)	3.53±1.08	3.27±0.85	0.004
Sampled sites	Before treatment	After treatment	p-Value
Visible plaque (%)	91.18±19.35	41.91±28.02	<0.001
Gingival bleeding (%)	58.82±35.82	10.29±16.42	<0.001
BOP (%)	94.12±18.52	19.60±8.44	<0.001
PPD (mm)	5.38±0.85	3.37±0.75	<0.001
CAL (mm)	5.38±1.18	4.44±0.88	<0.001

7 ANEXOS

7.1 Anexo 1 – Parecer da Comissão de Pesquisa da Faculdade de Odontologia

-UFRGS
Universidade Federal do Rio Grande do Sul — Faculdade de Odontologia

PARECER DA COMISSÃO DE PESQUISA

Parecer aprovado em reunião do dia 29 de abril de 2011. ATA 04/11.

A Comissão de Pesquisa da Faculdade de Odontologia, da Universidade Federal do Rio Grande do Sul, após análise aprovou o projeto abaixo citado por apresentar mérito científico, estar descrito adequadamente e possuir metodologias apropriadas.

PROJETO: Nº18917. O EFEITO DO CONTROLE DO BIOFILME SUPRAGENGIVAL E DA COMBINAÇÃO DO CONTROLE DO BIOFILME SUPRA E SUBGENGIVAL NA SAÚDE PERIODONTAL DE PACIENTES PARTICIPANTES DE UM PROGRAMA DE MANUTENÇÃO PERIODONTAL PREVENTIVA – UM ENSAIO CLÍNICO RANDONIZADO.

PESQUISADOR RESPONSÁVEL: Profª. SABRINA GOMES

OUTROS PESQUISADORES: Prof. Rui Vicente Opermann

Porto Alegre, 29 de abril de 2011.

Prof. Dr. Alex Nogueira Haas
Coordenador da Comissão de Pesquisa

7.2 ANEXO 2: Parecer do Comitê de Ética em Pesquisa da UFRGS

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comitê De Ética Em Pesquisa Da Ufrgs

PROPESQ

CARTA DE APROVAÇÃO

Comitê De Ética Em Pesquisa Da Ufrgs analisou o projeto:

Número: 18917
Título: O efeito do controle do biofilme supragengival e da combinação do controle do biofilme supra e subgengival na saúde periodontal de pacientes participantes de um programa de manutenção periodontal prev

Pesquisadores:

Equipe UFRGS:

SABRINA CARVALHO GOMES - coordenador desde 01/08/2011

Comitê De Ética Em Pesquisa Da Ufrgs aprovou o mesmo , em reunião realizada em 16/06/2011 - sala de reuniões ! do Gabinete do Reitor, 6º andar do prédio da Reitoria, por estar adequado ética e metodologicamente e de acordo com a Resolução 196/96 e complementares do Conselho Nacional de Saúde.

Porto Alegre, Quarta-Feira, 11 de Abril de 2012

BRUNO CASSEL NETO
Vice Pró-Reitor de Pesquisa

7.3 ANEXO 3: Termo de Consentimento Livre e Esclarecido

O efeito do controle do biofilme supragengival e da combinação do controle do biofilme supra e subgengival na saúde periodontal de pacientes participantes de um programa de manutenção periodontal preventiva. Um ensaio clínico randomizado.

Nome completo:_____

Idade_____ RG:_____Tel_____

1) Essa pesquisa tem o objetivo de verificar a importância da higiene bucal que realizamos todos os dias no resultado do tratamento de doenças da gengiva e na manutenção dos resultados desse tratamento. Nessa pesquisa, inicialmente, faremos exames clínicos para a seleção dos voluntários. Será realizada, também, uma entrevista, comum a qualquer atendimento odontológico, com perguntas relacionadas à sua saúde geral e a seus hábitos de higiene bucal. Os possíveis desconfortos relacionados a essa seleção são aqueles decorrentes de um exame de rotina e do tratamento da gengiva. Serão tomadas todas as medidas para garantir que o exame e tratamento sejam seguros, tais como uso de materiais descartáveis e de instrumentais esterilizados.
2) Após esses primeiros exames, você poderá ser selecionado ou não para o estudo. Se você não for selecionado, os benefícios associados à sua participação nesse exame são o acesso a um diagnóstico preciso da sua condição bucal, bem como esclarecimento e encaminhamento para tratamento, de acordo com as suas necessidades. Fica, ainda, assegurado o direito ao sigilo de todos os dados coletados, dos participantes selecionados ou não, sendo que, em nenhum momento, será permitido acesso de outra pessoa a esses dados, além do pesquisador ou do próprio indivíduo. Se você for selecionado, e tendo aceitado participar como voluntário será marcada nova data, a ser agendada pelo pesquisador responsável, para a realização de um exames clínicos e exames para avaliação da inflamação e infecção presentes. Esses exames são absolutamente seguros e são comuns aos tratamentos de gengiva.
3) A partir de sua inclusão no estudo, você será chamado a comparecer semanalmente, com horário agendado, para no mínimo 8 consultas, à clínica de periodontia (tratamento de gengiva) para receber os exames e tratamentos de gengiva necessários, bem como o acompanhamento adequado. O tratamento a ser realizado é conhecido, seguro e faz parte de todo os tratamentos de gengiva já existentes. Faz-se uma raspagem dos dentes (remoção do tártaro), polimento e instrução de higiene bucal. Esse tratamento resolve o problema de inflamação da gengiva (gengivite) e da doença chamada periodontite. Para esse tratamento será necessário anestesiar o local a ser tratado para diminuir desconfortos comuns a esses procedimentos. O tratamento da periodontite será realizado em todos os participantes. Uma vez terminada a fase inicial do tratamento você deverá comparecer uma vez a cada três meses durante dois anos para que se examine como as suas gengivas estão respondendo ao tratamento, avaliar como você esta cuidando da sua higiene bucal e eventualmente tratar a periodontite se ela se apresentar novamente. Nesse período de 2 anos você também terá a oportunidade de receber tratamento odontológico na Faculdade de Odontologia de acordo com as possibilidades de atendimento existentes.
4) Os conhecimentos adquiridos, com o presente estudo, serão importantes, futuramente, para a prevenção e tratamento das doenças de gengiva, pois poderão contribuir para que melhores formas de tratamento da gengivite e da periodontite sejam desenvolvidas. É importante ressaltar que toda e qualquer dúvida a respeito desse estudo será esclarecida pelo pesquisador responsável e que você poderá requisitar esse esclarecimento a qualquer momento. Esperamos poder atendê-lo em todas as suas dúvidas e nos comprometemos a dar todas as informações que você precisar ou que tornarem-se necessárias no decorrer do estudo.

5) Sua participação no estudo é voluntária e você, a qualquer tempo, pode afastar-se dele, sem que isso implique em qualquer prejuízo ao atendimento que você precisa. Nessa pesquisa a sua identificação (nome, RG, endereço, telefone) será confidencial. Somente os dados dos exames serão utilizados para fins de pesquisa, sem a identificação dos voluntários, preservando, assim, a sua privacidade. Eventualmente, fotos dos seus dentes e suas gengivas poderão ser interessantes para registrar as mudanças que ocorrerão com o tratamento. Quando isso for necessário, será solicitada sua permissão. Uma negativa sua não implicará em nenhuma alteração nos cuidados à sua pessoa. Por outro lado, se você concordar que as fotos sejam tomadas, fica garantido que não será possível, sob qualquer circunstância, a associação da foto com sua pessoa, garantindo-se assim absoluto sigilo da sua identidade. As escovas de dente a serem utilizadas pelos voluntários serão cedidas pelo pesquisador responsável pelo estudo. Não haverá ressarcimento dos prováveis gastos que o voluntário possa ter ao participar do presente estudo.

Qualquer sugestão, problema, dúvidas ou reclamações, ligue para nós:

Profa. Sabrina Carvalho Gomes (51)33085318 ou Comitê de Ética em Pesquisa: (51) 33083629

Por esse instrumento particular, declaro, para fins de *Ética e Legislação em Pesquisa*, que eu, _____, nascido (a) em ____/____/___,portador do RG_____ (Órgão Expedidor_____), residente à _____no. _____ Bairro _____, Cidade _____, Estado _____, que eu li e entendi as informações acima citadas e concordo em Participar da Pesquisa "O efeito do controle do biofilme supragengival e da combinação do controle do biofilme supra e subgengival na saúde periodontal de pacientes participantes de um programa de manutenção periodontal preventiva - Um ensaio clínico randomizado".

_____, _____de _____de 20____

Assinatura do voluntário _____

*Assinatura do pesquisador*_____

7.4 ANEXO 4: Ficha de entrevista dialogada

Dados pessoais

Nome:_____Identidade:_____

Endereço:_____

Cidade:_____Telefone: (_____)_____

Sexo: (_)F (_) M Data de Nascimento: ___/___/19___ Qual é a sua idade hoje?_____anos

A sua raça ou cor é: (_) Branca (_) Negra (_) Parda/Mulata (_) Amarela (_) Indígena

Você é: (_) solteiro (_) Casado/Vivendo com alguém (_) Divorciado (_) Viúvo (_) Outro_____

Você é alfabetizado: (_)Sim (_) Não Você estudou até: _____

Hábitos de Higiene Bucal

Com qual frequência você escova seus dentes:_____

Você divide a escova de dente com outras pessoas? (_) Sim (_) Não

O que você usa, frequentemente, para limpar entre os dentes? (_)Nada (_)Fio dental (_)Palito (_)Escova interdental (_)Outro_____

Se sim, com qual frequência?_____

Você usa algum produto para bochecho? (_)Não (_)Cepacol (_)Listerine (_)Plax (_)Outro_____

Se sim, com qual frequência?_____

Alguma vez alguém te ensinou a escovar os dentes? (_)ninguém (_)familiar (_)professora (_)dentista (_)outro_____

Quando foi a última vez que você visitou um dentista? (_)meses (_)anos (_)não lembra (_)nunca visitou

Você tem ido ao dentista nos últimos 5 anos? (_) quando tem dor, um dente quebrado ou outra urgência
(_) tem ido regularmente para fazer manutenção
De quanto em quanto tempo? _____
(_) não tem ido

Percepção das condições bucais e atitudes

Nos últimos 12 meses, você teve:

	Frequentemente	Algumas vezes	Raramente	Nunca
Mau hálito, mau cheio ou gosto ruim na boca				
Dor de dente				
Dentes frouxos				
Apertamento dental (ranger dentes)				
Sensação de boca seca				
Dor enquanto escova os dentes				
Feridas nas gengivas				
Sangramento nas gengivas				

Você acha que seus dentes da frente mudaram de posição com o passar dos anos? (_)Sim (_)Não

O que você faz quando sua gengiva sangra? (_)não sangra (_)nada/continua escovando normalmente (_)evita tocar onde sangra (_)escova com menos força/frequência (_) escova com mais força/frequência
(_) outra_____

Conhecimento

Você considera que sabe (_)muito (_)pouco (_)muito pouco (_)nada sobre doença da gengiva?

Em sua opinião, uma pessoa com_____está com doença da gengiva?

	Sim	Não	Não sei
dor na gengiva			
inchaço na gengiva			
sangramento da gengiva			
dente móvel ou frouxo			

Em sua opinião, _____ pode causar doença da gengiva?

	Sim	Não	Não sei
escovar os dentes de forma incorreta			
mais de uma pessoa usar a mesma escova			
fumar cigarros			
tártaro nos dentes			
herdar a doença dos pais			
possuir dentes mal posicionados ou tortos			
bactérias e germes			

Fatores comportamentais

Você fuma atualmente? (_) Não (_)Sim -----> Quanto cigarros por dia?_____ Há quantos anos?_____

Você fumou anteriormente? (_)Não

(_)Sim ------> Quantos cigarros por dia?____ Por quantos anos?_____

Quanto tempo faz que você parou de fumar?_____

Você toma chimarrão? (_)frequentemente (_)às vezes (_)raramente (_)nunca

Você ingere bebidas alcoólicas? (_)frequentemente (_)às vezes (_)raramente (_)nunca

Se sim: Qual tipo? (_)cerveja (_)vinho (_)cachaça (_)outros_____

Quantas doses/copos você, em geral, ingere por semana?_____

Fatores psicossociais

Nos últimos 3 anos você teve:

	Sim	Não	Não sei
um problema sério de saúde?			
um problema sério de saúde na sua família?			
morte de um membro próximo da família?			
algum outro problema que tenha afetado você emocionalmente de forma muito séria?			

Em relação a seu presente trabalho:

Quantas horas por semana você trabalha? ____horas (_)desempregado (_)aposentado/estudante/do Lar

Você esteve desempregado por mais de 3 meses nos últimos 3 anos? (_)sim (_)não

Se esteve, por quanto tempo?_____ meses

Você acha que os ganhos mensais da sua família: (_)não são suficientes para pagar as contas

(_)apenas suficientes para pagar as contas

(_)suficientes para pagas as contas e economizar

Você considera a qualidade da sua vida: (_)muito ruim (_)ruim (_)razoável (_)boa (_)muito boa

Nível socioeconômico

Quanto você recebe por mês:_____

Quantas _____você possui?

	Não tem	1	2	3	4 ou mais
TVs coloridas					
Rádios					
Banheiros					
Automóveis					
Empregadas (pagas mensalmente)					
Aspiradores de pó					
Máquinas de lavar roupa					
Dvds/Videocassetes					
Refrigeradores					
Freezer (considerar refrigerador duplex)					

Quantas pessoas você sustenta economicamente? ___ pessoas (além de você e pessoas c/ renda própria)
Quantas pessoas moram com você?____pessoas (além de você mesmo)

História médica

Você tem:

	Sim	Não	Não sei
Diabetes?			
Asma, alergia a alimento, pós, etc?			
Infecções respiratórias recorrentes (3 ou + por ano)?			
Doença cardíaca ou arterial?			
Artrite reumatoide?			

Outro problema de saúde?_____
Você está usando alguma medicação? (_)Não (_)Sim --->Qual?_____
Para participantes mulheres,
Você está na menopausa? (_)Não (_)Sim --->Você está realizando reposição hormonal? (_)Não (_)Sim

Crenças

Em sua opinião ____ é____ para se ter uma boca saudável.

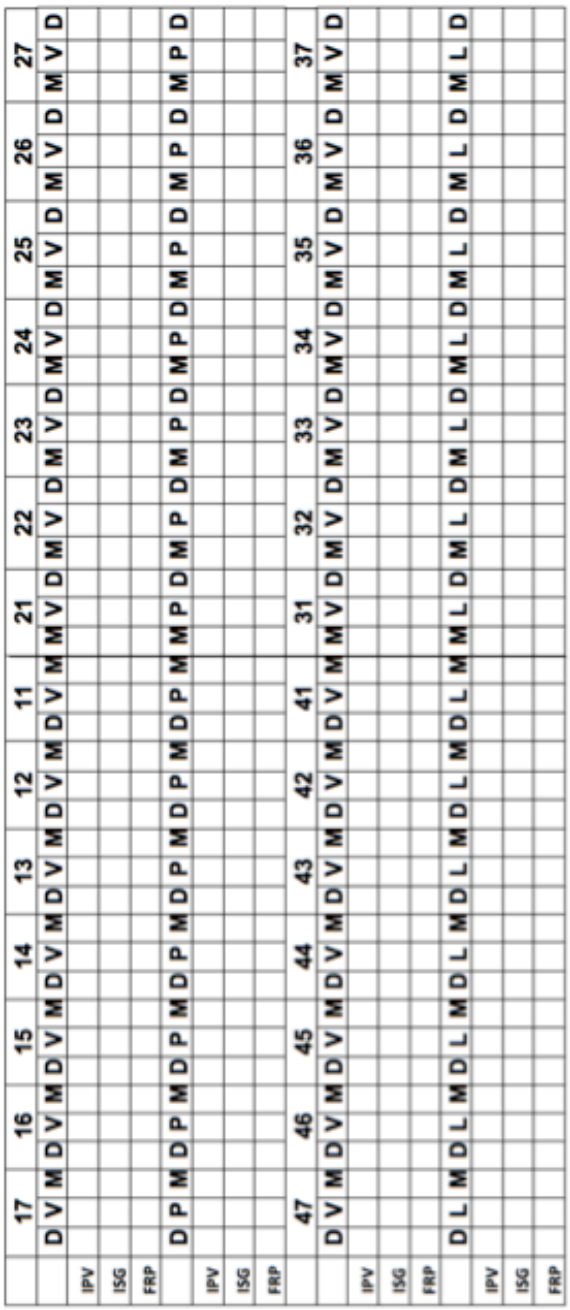
	Muito importante	Importante	Pouco importante	Sem importância
escovar os dentes regularmente				
usa palitos de dente e/ou fio dental				
evitar dividir escovas de dente				
evitar fumar cigarros				
evitar o uso excessivo de açúcar				
visitar regularmente o dentista				
usar pasta de dentes com flúor				

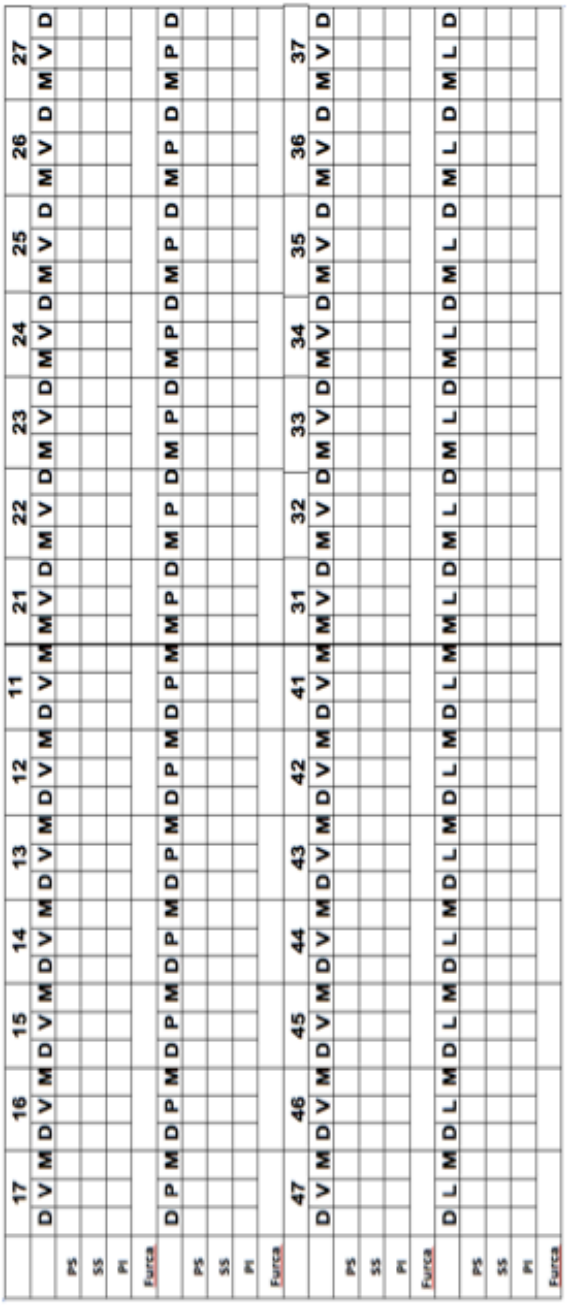
Hereditariedade

Alguns dos seus pais têm ou tinham:

	Sim	Não	Não sei
diabetes?			
asma, alergia a alimentos, pó, etc?			
infecções respiratórias repetidas (3 ou + por ano)?			
doença cardíaca ou arterial?			
doença de gengiva?			

7.5 ANEXO 5: Ficha de coleta de dados clínicos





7.6 ANEXO 6: Ficha de coleta de dados imunológicos

Coleta Imunológica 1: 8 maiores PS por paciente – exceto dentes com lesão de furca/endo-perio.

	Rasos				Profundos			
	Sítio 1 _____	Sítio 2 _____	Sítio 3 _____	Sítio 4 _____	Sítio 5 _____	Sítio 6 _____	Sítio 7 _____	Sítio 8 _____
IPV								
ISG								
PS								
SS								
PI								
UP								
Eppendorf								

Coleta Imunológica 2

	Rasos				Profundos			
	Sítio 1 _____	Sítio 2 _____	Sítio 3 _____	Sítio 4 _____	Sítio 5 _____	Sítio 6 _____	Sítio 7 _____	Sítio 8 _____
IPV								
ISG								
PS								
SS								
PI								
UP								
Eppendorf								

Coleta Imunológica 3

	Rasos				Profundos			
	Sítio 1 _____	Sítio 2 _____	Sítio 3 _____	Sítio 4 _____	Sítio 5 _____	Sítio 6 _____	Sítio 7 _____	Sítio 8 _____
IPV								
ISG								
PS								
SS								
PI								
UP								
Eppendorf								

Coleta Imunológica 4

	Rasos				Profundos			
	Sítio 1 _____	Sítio 2 _____	Sítio 3 _____	Sítio 4 _____	Sítio 5 _____	Sítio 6 _____	Sítio 7 _____	Sítio 8 _____
IPV								
ISG								
PS								
SS								
PI								
Unidades de Periotrom								
Eppendorf								

7.7 ANEXO 7: Orientações aos pacientes

Orientações

Prezados participantes,

Solicitamos sua atenção para as seguintes orientações a serem seguidas durante o estudo:

13. **Se necessário** tomar alguma medicação anti-inflamatória ou antibiótica COMUNIQUEM-NOS comunique imediatamente, antes de iniciá-la (81279227 ou 33085318).
14. NÃO utilize nenhum creme dental ou escova que não sejam aqueles fornecidos pela equipe.
15. Lembre-se de não compartilhar o creme e escova com ninguém, pois todos eles serão pesados e analisados após o uso.
16. O (a) senhor (a) DEVRÁ TRAZER O CREME DENTAL E A ESCOVA EM TODAS AS CONSULTAS
17. NÃO use nenhum produto para bochecho, nem mesmo aqueles caseiros (chás, etc)
18. Não é necessário fazer nenhum outro tratamento odontológico neste período. As necessidades serão avaliadas e encaminhadas para tratamento dentro da Faculdade de Odontologia da UFRGS.

7.8 ANEXO 8: Ficha de acompanhamento do tratamento

Descrição do Tratamento Periodontal

* anotar todos os aspectos do tratamento realizados a cada sessão, incluindo principalmente as PERDAS DENTÁRIAS e sua razão, destacando-as no texto.

Data	Procedimento	Responsável