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RHEUMATOID ARTHRITIS AS A MODIFIER OF PERIODONTITIS

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To the memory of my mormor ***Yara***

ABSTRACT

Periodontitis is a chronic tissue-destructive condition in which the tooth-supporting collagen fibers of ligament and bone are broken down, mainly by the host's overreactive immune inflammatory response. The relation between periodontitis and other chronic inflammatory destructive diseases, such as rheumatoid arthritis (RA), has been dealt with in some studies because, in spite of their different etiologies, similar mechanisms of tissue destruction have been described in these conditions. The findings concerning the periodontal conditions of adults with RA are disputed. Some studies have shown no association between the two conditions while others have supported a worse periodontal status in these patients. Little is known about the oral conditions of individuals with the forms of arthritis that affect children and adolescents, *i.e.* juvenile idiopathic arthritis (JIA), except for a higher caries prevalence. Information regarding periodontitis in JIA subjects is lacking and thus is needed.

The **aims** of this thesis were to assess the periodontal conditions of adolescents with JIA relating this to their rheumatological status, to investigate the possible effect of their aberrant inflammatory response on serum and gingival crevicular fluid (GCF) markers of inflammation and subgingival microbiota, to monitor changes in periodontal inflammation in these individuals for 2 years after continuous rheumatological treatment and finally to evaluate the effect of anti-rheumatic medication on markers of periodontal inflammation in GCF from a group of individuals with RA. **Study I** showed that adolescents with JIA present more frequently incipient periodontal attachment loss than healthy controls, in spite of similar plaque and marginal bleeding levels. Individuals with JIA with attachment loss (AL) tended to have higher levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and joints with swelling, pain and limitation on movement. **Study II** demonstrated that serum levels of interleuin-1β (IL-1β) and interleukin-18 (IL-18) were significantly elevated in the JIA group, especially in those presenting AL, suggesting that the early periodontal destruction observed in these patients might be related to their altered systemic inflammatory response. In **Study III**, the clinical and laboratory rheumatological parameters were significantly improved after 2 years of follow-up. Accompanying this, the total amounts of IL-1β decreased in GCF and no differences were observed in periodontitis parameters. In **Study IV,** total amounts of IL-1β and total elastase were significantly lower in an adult RA group compared to

matched controls. These markers were significantly correlated in the RA group. The heavy anti-inflammatory treatment taken by RA patients might influence the periodontal inflammatory status in GCF represented by IL-1β and elastase.
In conclusion, this thesis shows that adolescents with JIA, especially those more systemically affected, have a worse periodontal condition than controls. However, longitudinally, the effects of disease remission and anti-rheumatic treatment are potentially able to modulate the inflammatory process in the periodontium.

Keywords: periodontal disease, attachment loss, juvenile idiopathic arthrtitis, rheumatoid arthritis, interleukin-1β, interleukin-18, elastase

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LIST OF PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their roman numbers (I-IV):

I. Miranda L.A., Fischer R.G., Sztajnbok F.R., Figueredo C.M.S., Gustafsson A. Periodontal conditions in patients with juvenile idiopathic arthritis. *Journal of Clinical Periodontology* 2003; 30: 969–974.

II. Miranda L.A., Fischer R.G., Sztajnbok F.R., Johansson A., Figueredo C.M.S., Gustafsson A. Increased interleukin-18 in patients with juvenile idiopathic arthritis and early attachment loss. *Journal of Periodontology* 2005;76:75-82.

III. Miranda L.A., Braga F., Fischer R.G., Sztajnbok F.R., Figueredo C.M.S., Gustafsson A. Changes in periodontal and rheumatological conditions after 2 years in patients with juvenile idiopathic arthritis. *Journal of Periodontology* 2006;77:1695-1700.

IV. Miranda L.A., Islabão A.G., Fischer R.G., Figueredo C.M.S., Oppermann R.V., Gustafsson A. Decreased IL-1β and elastase in the GCF of individuals with rheumatoid arthritis. *Submitted.*

CONTENTS

LIST OF ABBREVIATIONS

A1AT	α-1-antitrypsin
A2MG	α-2-macroglobulin
ABS	Absorbance
AL	Attachment loss
CAL	Clinical attachment level
CHAQ	Children health assessment questionnaire
CRP	C-reactive protein
DMARD	Disease modifier anti-rheumatic drug
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
GCF	Gingival crevicular fluid
HAQ	Health assessment questionnaire
IL-1β	Interleukin-1β
IL-18	Interleukin-18
ILAR	International League of Associations for Rheumatology
JIA	Juvenile idiopathic arthritis
JIA AL	Juvenile idiopathic arthritis with attachment loss
JIA NoAL	Juvenile idiopathic arthritis without attachment loss
LOM	Limitation on movement
mABS	Milliabsorbances
MMP-8	Matrix metalloproteinase-8
MTX	Methotrexate
NSAID	Non-steroidal anti-inflammatory drug
PatGA	Patient's global assessment
PCR	Polymerase chain reaction
PD	Probing depth
PGA	Physician's global assessment
PGE_2	Prostaglandin E_2
RA	Rheumatoid arthritis
TIMP	Tissue inhibitor of metalloproteinase
TNF-α	Tumour necrosis factor-α

INTRODUCTION

The view that periodontitis is a chronic inflammatory reaction towards the dental biofilm challenge with a destructive nature has been widely discussed in the periodontal literature (Figueredo 1999; Graves & Cochran 2003; Teng 2006a; Teng 2006b; Van Dyke & Serhan 2003). Under this prism, it is believed that tissue destruction of the supporting periodontal tissues is mediated by an overreactive immune inflammatory response to bacteria in the subgingival environment. In individuals who do not have this altered host response, periodontal inflammation remains non-destructive, as a gingivitis. In other words, the susceptibility of each individual reflected in the nature of the immune inflammatory response determines the destructive character of the disease (Gemmell et al. 2002; Taubman et al. 2005). Besides, the complex composition of the dental biofilm and genetic, systemic and behavioural factors concur to a protective or a destructive nature of the immune inflammatory response (Page et al. 1997).

Similar to other chronic tissue-destructive conditions, the process of tissue destruction in periodontitis is mainly associated to the action of the innate immune system, even though this system influences and is influenced by the adaptive response (Abbas et al. 2000). In simple terms, tissue destruction occurs by the stimulatory action of pro-inflammatory cytokines, the direct action of oxygen free radicals and proteolytic enzymes released by neutrophils, monocytes/macrophages and tissue resident cells and the action of bone resorption mediators, all these processes under the regulation of B and T cells (Gemmell & Seymour 2004; Han et al. 2006; Kawai et al. 2006). Even though bacterial proteases may be involved in the periodontal destruction process, it is believed that the mayor part of tissue destruction in periodontitis is caused by mediators of the immune inflammatory response (Page et al. 1997; Teng 2003).

The similar tissue destruction mechanisms observed in periodontitis and other chronic inflammatory diseases, such as rheumatoid arthritis (RA), inflammatory bowel disease and glomerulonephritis, has been dealt with in some studies (Engel et al. 1988; Flemmig et al. 1991; Grossner-Schreiber et al. 2006; Kupari & Teerenhovi 1981). Of these possible associations, that with RA has been the most studied. Both periodontitis and RA are characterized clinically by the local destruction of hard and soft tissues due

to the host immune inflammatory response and similarities in their natural history, immunogenetics and immunopathology have been recently revised (Bartold et al. 2005). Although some earlier studies associating both conditions show conflicting results, other recent evidence shows that RA patients have a poorer periodontal status (Mercado et al. 2001; Sjostrom et al. 1989; Tolo et al. 1991).

The oral conditions, in general, of individuals having the type of arthritis that affects children and adolescents, *i.e.,* juvenile idiopathic arthritis (JIA) are regarded as compromised or poor (Walton et al. 2000). There are observations of higher plaque and gingivitis levels and higher caries prevalence in these individuals (Siamopoulou et al. 1989; Welbury et al. 2003). However, information regarding periodontal destructive conditions in subjects with JIA was lacking. Recently, Reichert et al. (2006) and Havemose-Poulsen et al. (2006) evaluated in more detail the periodontal conditions of patients affected by this rheumatic disease.

RHEUMATOID ARTHRITIS AND JUVENILE IDIOPATHIC ARTHRITIS

RA is a complex systemic inflammatory disease primarily affecting the joints characterized by synovitis and abnormal immune response. RA is very often accompanied by extra-articular inflammatory manifestations and its prevalence among adults is approximately 1%, affecting mainly women (Scott & Kingsley 2006). Juvenile idiopathic arthritis (JIA) refers to a group of diseases that have in common chronic arthritis of no known cause and various systemic manifestations, usually starting before the age of 16 years (Petty et al. 1998). It is considered to be the main disorder of connective tissue in childhood and adolescence, with a prevalence ranging from 0.07 to 4.01 per 1000 children and an annual incidence of 0.226 per 1000 children (Manners & Bower 2002). Nowadays JIA is classified in 8 subtypes according to the International League of Associations for Rheumatology (ILAR) (Petty et al. 2004). Long-term studies have showed that less than half of the patients have active disease at a 16.5-years follow-up, usually at a low level (Minden et al. 2002) and with remission rates ranging from 45 to 60% (Foster et al. 2003; Koivuniemi & Leirisalo-Repo 1999; Minden et al. 2002).

Even though JIA presents some clinical and genetic differences from RA in adults, both are considered heterogeneous syndromes characterized by progressive joint inflammation leading to articular and periarticular tissues destruction and subsequently, physical and quality of life impairments (Choy & Panayi 2001). Both conditions have a marked autoimmune involvement (Chini et al. 2002; Wedderburn & Woo 1999). Synovial lesion and synovial fluid content are indistinguishable (Cassidy 1986). Additionally, in both conditions, systemic signs of inflammation, such as increased levels of acute-phase proteins in serum are elicited (Gonzalez-Gay et al. 2005; Hussein et al. 1987).

From the rheumatological point of view, the clinical assessment of disease activity and severity in RA and JIA is usually based on various measurements. These include the number of joints with pain, swelling and limitation of movement (LOM) and the total number of joints involved, subjective variables – e.g., the physician's, parent's or patient's perception of symptoms, measures of functional capability i.e., the Health Assessment Questionnaire (HAQ) and its children's version (CHAQ) – and laboratory indicators of inflammation, such as the erythrocyte sedimentation rate (ESR) and the capsule-reactive protein (CRP) (Ravelli et al. 1997).

The cause of RA and JIA is unknown, but it is likely that, in genetically predisposed individuals, an infective agent or another stimulus binds to toll-like receptors on peripheral dendritic cells and macrophages. This triggers a rapid response by the innate immune system involving cytokines and other inflammatory mediators, complement, natural killer cells, and neutrophils. Dendritic cells then migrate to lymph nodes, where they activate the adaptive immune system by presenting antigen to T cells. These activated T cells proliferate and migrate into the joint, where they stimulate a multimolecular immune–inflammatory cascade. T cells produce interferon-γ and other proinflammatory cytokines, which stimulate macrophages, fibroblasts, chondrocytes, and osteoclasts. Activated macrophages and fibroblasts release tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-15 (IL-15), interleukin-18 (IL-18), and other proinflammatory cytokines that stimulate the production of additional inflammatory mediators (chemokines, prostaglandins), proteases, and growth factors and activate neutrophils, B cells, and endothelial cells. Finally, joint damage, associated with the development of a locally

invasive pannus tissue, occurs through the actions of proteases and activated osteoclasts (Firestein 2003; Firestein 2005; Scott & Kingsley 2006).

Treatment of RA and JIA is typically initiated with nonsteroidal antiinflammatory drugs (NSAIDs) and simple analgesics to relieve pain and stiffness. DMARDs, which improve symptoms and reduce erosive damage, are initiated as early as possible. Standard conventional DMARDs include methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, and cyclosporine. Methotrexate is the most widely used. There is increasing emphasis, especially in severe disease, on using combinations of two or more conventional DMARDs. Steroids are often used to manage disease flares (Guidelines 2002; Scott & Kingsley 2006; Wallace 2006).

PERIODONTAL CONDITIONS OF INDIVIDUALS WITH RHEUMATOID ARTHRITIS

Regarding adults with RA, to date there is no reliable information on the impact of RA on periodontal conditions. Therefore, we conducted a systematic approach to review the evidence from clinical trials and observational studies on whether RA is associated with a worse periodontal condition. The focused question was: "What is the effect of RA on the periodontal conditions?"

Criteria for considering studies for this review

Types of studies

For this review, clinical trials, cohort, case-control and cross-sectional studies that have compared periodontal conditions of RA patients against systemically healthy controls, were searched.

Types of participants

Participants were subjects diagnosed with RA.

Types of outcome measures

We considered the following primary outcomes: a) tooth loss; b) clinical attachment level (CAL); c) recession; d) alveolar bone loss. We also considered the following secondary outcomes: a) probing depth, b) bleeding on probing, c) plaque levels and d) marginal bleeding levels.

Search strategy for identification of studies

The studies were identified from the following sources: MEDLINE (1966 to 2006), EMBASE (1974 to 2006) and LILACS (1982 to 2006). There were no restrictions on language or date. The keywords *rheumatoid arthritis, periodontal disease, periodontitis, gingivitis* were searched as text words and medical subject heading (Mesh) terms. The latest search for evidence for the systematic review and for the thesis as a whole was done in 12th December 2006. The reference lists of relevant papers were checked for any study that may have been overlooked.

Methods of the review

Locating and selecting the studies

One reviewer assessed the titles and abstracts of all reports of trials identified by the electronic searching without blinding to authorship. Full text hard copies were obtained for studies that appeared to fulfill the selection criteria. A list of the selected papers and duplicate sets of the papers were made available to the reviewers for their independent analyses. Resolution of discrepancies was done by consensus. Studies were assessed to ascertain that they met the inclusion criteria according to type of study, type of participants and type of outcomes.

Critical appraisal of selected trials

The methodological quality of each study was assessed by two reviewers and carried out using the guidelines provided by the MOOSE statement (Stroup et al. 2000). The quality of the observational studies identified was evaluated according to the study setting, completeness and duration of follow-up, validity and completeness of exposure and outcome ascertainment, comparability of the control group and adjustment for known confounding variables (Singh-Grewal et al. 2005).

The internal validity of each study was assessed according to presence of systematic bias (selection, measurement, confusion).

The external validity of each study was assessed by characteristics of the participants and the outcomes.

When the reviewers were unable to make a decision about the classification of a study due to lack of information on it, they tried to contact the authors.

Collecting data

The same two reviewers working independently extracted data and the results were cross-checked for accuracy. The characteristics and results of each study were

summarized in tables. Data from the study with multiple publications were extracted from the more complete publication.

The studies were described by the following characteristics:

1) Methods: length of follow-up period, masked assessment of primary outcomes, baseline assessment of primary outcomes, reliable primary outcomes measure(s), protection against contamination, training, setting (location of care, academic status, country), unit of analysis, power calculation, sample representativeness.

2) Participants: inclusion criteria, exclusion criteria, duration of disease (years since the diagnosis of RA), gender, severity of RA, smoking status, and other co-morbidities, proportion of eligible participants, number of participants included in the study.

3) Outcomes: primary outcomes(s) definition and differences among them.

4) Notes: sources of funding, ethical approval, conflict of interest of the authors.

Analysing and presenting results

Tables and a narrative synthesis are provided.

Literature search

The study selection process is outlined in Figure 1. From 38 titles and abstracts retrieved, 17 had the full-text retrieved and 10 fulfilled the inclusion criteria. One study was found in duplicate, finally resulting in 9 studies included in the systematic review.

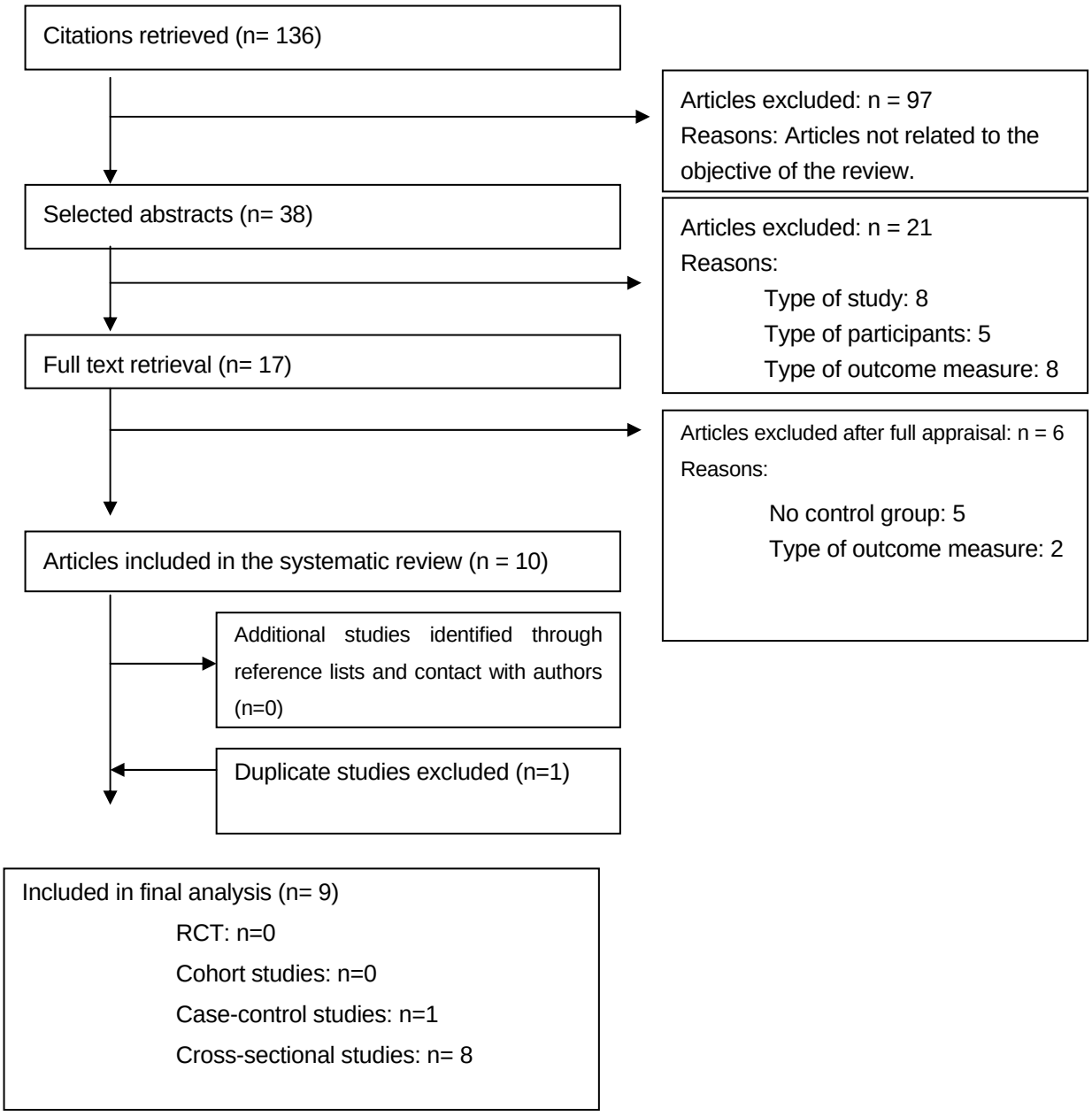

Figure 1. Flow chart outlining the study selection process for the effect of RA on the periodontal conditions.

Characteristics of the studies included

The characteristics and actual outcomes of the studies included are outlined in Table 1. These studies were published between 1989 and 2005 and all of them are observational

studies: 8 cross-sectional and 1 case-control. The country of origin varies, 2 from Australia, 2 from Turkey, 2 from from Norway, 1 from Egypt, 1 from Germany and 1 from Sweden. Regarding the study setting, only 1 was done in a private practice and university clinics, the others, in university settings. Most of them compared the periodontal conditions of patients with RA to healthy controls. One study had a third group of periodontitis patients (Bozkurt et al. 2000). The mean age of participants ranged from 42.3 to 56.4 years old and most of them were women. A variety of outcome measures were employed: primary (considered as such for this review) tooth loss – 7/9 studies, CAL – 3/9 studies, recession – none, alveolar bone loss – 3/9 studies; secondary: probing depth – 6/9 studies, BOP – 1/9 studies, plaque levels – 4/9 studies, gingivitis levels – 5/9 studies.

Quality of the included studies

The quality assessment of the studies includes items related to methods, participants, results and other notes and is summarized in Table 2. When considering number of remaining teeth or tooth loss, 4 studies found significantly less teeth present in RA patients compared to controls and 3 do not corroborate these findings. Regarding CAL, different ways of analyzing this measure were employed. Abou-Raya et al. (2005) presented data on the prevalence of individuals with CAL> 4 mm, which was significantly higher in RA patients than in controls. Bozkurt et al. (2000) and Gleissner et al. (1998) reported mean values of CAL. In the former study, no difference was found between the groups RA and periodontitis compared to controls and in the later, RA patients had mean CAL significantly higher than controls. Bone loss was evaluated in different forms as well. Mercado et al. (2001) used categories of the Jordan and Hugoson classification. These authors found that more RA patients had moderate to severe bone loss than controls. Tolo et al. (1990) measured loss of approximal bone expressed as a percentage of total root length and found statiscally more bone loss in the RA group. Sjotrom et al. (1989) measured the alveolar bone level as a percentage of the total length of the tooth and observed no difference between groups.

Plaque levels were found to be similar between RA and controls in 2 studies (Mercado et al. 2001, Gleissner et al. 1998); 1 study found more plaque in RA individuals (Bozkurt et al. 2000) and 1 study found less plaque in this same group (Sjotrom et al. 1989). For gingivitis, 3 studies found that RA subjects had more gingival inflammation than controls and 2 found no difference between groups. With respect to PD, Abou-

Raya et al. (2005) and Mercado et al. (2001) reported significantly more RA individuals having deeper pockets than controls. This finding is not corroborated by Sjotrom et al. (1989) who found similar prevalence for these groups. The other 3 studies that evaluated PD used mean values and 2 found a higher mean PD for the RA group (Bozkurt et al. 2000; Gleissner et al. 1998) and 1 found a lower mean (Yavuzyilmaz et al. 1992). Only one study contemplated BOP and no difference was observed between RA and controls (Mercado et al. 2001).

The studies included in the present review show a substantial variation in the results found. Taking into account the primary outcome measures considered adequate by this review, of the 9 studies included, 4/7 found more tooth loss in RA patients, 2/3 found more CAL and 2/3 found more bone loss. A worse periodontal condition in individuals with RA is difficult to be confirmed by this review based on the available data. Mainly, there is no evidence stemming from other systematic reviews, clinical trials and longitudinal studies. In addition, the studies selected present considerable methodological limitations.

Important issues such as blinding and training of the examiners have not been employed in most of the studies. In the Mercado et al. (2001) study, one of the radiographs examiners was not aware of the RA status of the subjects. This study and Gleissner at al. (1998) showed data regarding calibration of the examiners. Arneberg et al. (1992) used questionnaires and applied reliability and validity tests to confirm the self-reported information.

Regarding appropriate sample sizes, none of the studies reported power calculation. Only Sjostrom et al. (1989) used the term *statistical sample*. However, no details on calculation of sample size have been reported.

With respect to the origin of the samples, most studies used convenience samples. Individuals with RA were selected at rheumatology units and controls usually at general dentistry clinics. Only Sjostrom et al. (1989) used a population-based sample from the borough of Jonköping, Sweden. Selection of controls is critical in observational studies and dental settings should be avoided to diminish selection biases. All studies attempted to match the exposed and non-exposed groups for age and most of them for sex. Two studies also matched the groups for other important variables associated with periodontitis such as social status, oral hygiene and smoking (Gleissner et al. 1998; Abou-Raya et al. 2005). Only Arneberg et al. (1992) compensated this adjusting for smoking habits, dental attendance, social class and caries problems. Smoking is considered a risk factor for

periodontitis (Susin et al. 2004) and was not accounted for in 5 of the 9 studies reviewed, reflecting in an important limitation of the data available.

The diagnostic criteria for RA employed in all studies were that of the American College of Rheumatology of 1987, the most used in Rheumatology nowadays. The duration and severity of RA varied considerably between studies. Seven from 9 studies reported on the disease duration, ranging from 1 year to 42 years. The severity of RA was reported by 3 studies. The longer the exposure time to RA and more severe the disease, the more expected is the appearance of the influence on the conditions of the periodontium.

The effect of medications used to treat RA, such as NSAIDs and DMARDs, has to be considered when evaluating the periodontal conditions of RA subjects. Although matching or adjusting for medication use would be essential, this issue was reported in detail only in the studies of Bozkurt et al. (2000), Gleissner et al. (1998) and Tolo et al. (1990).

The principal weakness of the present review is that it is dominated by cross-sectional studies of variable quality. Thus, definite conclusions regarding the periodontal conditions of individuals with RA could not be reached. More consistent studies, especially case-control and longitudinal ones, fulfilling key methodological principles are needed in order to clarify the effect of RA on the periodontal conditions.

Table 1. Characteristics of the studies included.

Reference/Year	Study design	Country	N	Age , years	Actual Outcome
Abou-Raya et al. 2005	Cross-sectional	Egypt	RA=50 CTR=50	RA: 48±10.8 CTR: 49.4±10.5	**Missing teeth:** RA:32%, CTR: 0%, p<0.001; **CAL>4 mm**: RA56%, CTR: 2%, p<0.001; **Gingivitis**: RA 88%, CTR: 38%, p<0.001; **PD>4mm:** RA: 72%, CTR:38%, p<0.001.
Mercado et al. 2001	Cross-sectional	Australia	RA=65 CTR=65	Whole group (n=130):56.4±11.6	**Missing teeth:** RA: 11.6, CTR: 6.7, p=0.000; **Bone loss** (in cathegories of Hugoson and Jordan classification: P0: none; P1: 1/3, mild; P2: 2/3, moderate; P3: >2/3, severe)**:** RA: 30.8%P0/P1*, 69.2% P2/P3*, CTR: 66.2% P0/P1, 33.8% P2/P3; **BOP:** RA: 31.3%, CTR: 30%, p=0.65; **Plaque**: RA: 23.9%, CTR: 24.3%, p=0.82; **PD** (in cathegories - PD0:2 deepest sites with 0-3mm; PD1: 3.2-6mm; PD2: 6.2-8mm; PD3: > 8.2mm**:** RA: 44.6%PD0/PD1*, 55.4% PD2/PD3*, CTR: 75.4%PD0/PD1 24.6% PD2/PD3.
Bozkurt et al. 2000	Cross-sectional	Turkey	RA=15 CTR=15	RA: 47.80±6.5 CTR: 45.67±7.2	**CAL:** RA: 4.6, CTR: 1.8; **GI:** RA: 0.9, CTR: 0.2 **Plaque index:** RA: 2.4*, CTR: 1.8; **PD:** RA: 3.6, CTR: 1.8 **GCF IL-6 (pg/ul):** RA: 5.9, CTR: 3.8
Mercado et al. 2000	Case-control	Australia	PG=809 GG=603	PG:44.32 GG: 42.28	**Prevalence of RA in PG:** 3.95% **Prevalence of RA in GG:** 0.66% (p<0.05)
Gleissner et al. 1998	Cross-sectional	Germany	RA: 50 CTR: 101	RA: 53.4±9.6 CTR: 54.3±11.1	**Missing teeth:** RA: 7.2*, CTR: 5.0; **CAL**: RA: 2.5*, CTR: 1.0 **SBI (%):** RA: 65.6*, CTR: 44.4; **API (%):** RA: 72.9 , CTR:75.4, **PD:** RA: 2.9*, CTR:2.3
Arneberg et al. 1992	Cross-sectional	Norway	RA: 138 CTR: 50	RA: 44-56 CTR: 44-56	**Remaining teeth:** RA: 24, CTR: 25 (SD NR)
Yavuzyilmaz et al. 1992	Cross-sectional	Turkey	RA: 11 CTR: 11	RA: 42.63±1.62 CTR: 43.86±1.55	**Missing teeth:** RA: 12 CTR:11.45 **GI:** RA: 1.58*, CTR: 2.05 **PD:** RA: 3.17*, CTR:3.74
Tolo et al. 1990	Cross-sectional	Norway	RA: 37 CTR:37	RA: NR CTR: NR	**Number teeth:** RA: 17.9*, CTR: 22.6 **Bone loss:** RA: 23.8*, CTR: 18.9
Sjostrom et al. 1989	Cross-sectional	Sweden	RA: 161 CTR: 122	RA: NR CTR: NR	**Number teeth:** RA: 20, CTR:21; **Bone loss:** RA: 58, CTR: 57 **Gingivitis:** RA: 34%, CTR: 36% ; **Plaque**: RA: 38%*, CTR: 47% **Supra calculus:** RA: 65%, CTR: 75% ; **Sub calculus:** RA: 52%, CTR: 58% **PD≥4mm:** RA: 81% subjects, CTR: 76% subjects ; **PD≥7mm:** RA: 11%, CTR: 19%

RA: rheumatoid arthritis; CTR: controls; PD: probing depth; CAL: clinical attachment loss; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; BOP: bleeding on probing, GI : gingival index ; GCF: gingival crevicular fluid; PG: periodontitis group; GG: general group; SBI: sulcular bleeding index; API: approximal plaque index ; *statistically significant difference ; SD NR : standard deviation non reported.

Table 2. Quality of the studies included.

References / Quality criteria	Abou-Raya et al. 2005	Mercado et al. 2001	Bozkurt et al. 2000	Mercado et al. 2000	Gleissner et al. 1998	Arneberg et al. 1992	Yavuzyilmaz et al. 1992	Tolo et al. 1990	Sjostrom et al. 1989
Blinding	NR	Partial	NR	NR	NR	NA	NR	Yes	NR
Comparability of controls	Age, gender, social status, dental hygiene	Age, gender and smoking status	NR	Age	Age, gender, smoking and oral hygiene	Age	Age, gender and plaque levels	Age and gender	Age and gender
Adjustment for confounders	No	NR	NR	NR	NR	Yes	NR	Yes	NR
Duration of disease (RA)	5.5± 2.7 years	NR	1to 8 years	NR	12.9±7.9 years	4-9 ys: 33/125 10-19ys: 51/125 20-42ys: 41/125	NR	Mean of 13 years	1 to 28 years, mean of 13 years
Severity of disease (RA)	Duration of morning stiffness No. swollen joints VAS HAQ	Tenderness and swelling PGA Morning stiffness HAQ	NR	NR	Number of joints affected	Physical dysfunction score	NR	NR	NR
Gender	RA: 40F/10M CTR: 40F/10M	Reported for the whole group (n-130): F 74.6% M 25.4%	RA: 9M/6F CTR: 8M/7F PD: 11M/4F	NR	RA: 11M/39F CTR: 22M/79F	RA: 21M/104F CTR: 50F	RA: 5M/6F CTR: 5M/6F	NR	RA: 42M/119F CTR: 42M/80F
Smoking	Smokers excluded	RA: 7/65 smokers	NR	NR	RA: 41/50 CTR: 80/101	RA Daily	NR	NR	NR

status		CTR: 7/65 smokers				smoker:57/125 Not daily smoker:68/125 CTR: NR			
Use of medications	NR	NR	RA: Prenidsolone 5 mg/day Indomethacin 75 mg/day Chloroquine 250 mg/day	NR	RA: DMARDs: 46/50 Corticoids:39/50 NSAIDs: 42/50 DMARDs had been used for a mean time of 80 ±76 months (n=46), corticoids for 81 ± 76 months (n=39) and NSAIDs had bean taken for 80 ± 75 months (n=42).	RA: Use of a mean of 4.6 RA-medications for >1yr. CTR: NR	RA: anti-inflammatory drugs for extended periods, non-specified	RA NSAIDs: 37/37 MTX: 11/37 All patients had received anti-inflammatory drugs for extended periods and 11 patients had also received cytostatics (Prednisone, Imuran, Methotrexate)	RA: 86% taking NSAIDs, some with chloroquine

NR: non reported; NA: non applicable; RA: rheumatoid arthritis; CTR: controls; ys: years; VAS: visual analogue scale; HAQ: health assessment questionnaire; PGA: patient global assessment; F: female; M: male; PD: periodontitis group; DMARD: disease modifier anti-rheumatic drug; NSAID: non steroidal anti-inflammatory drug.

PERIODONTAL CONDITIONS OF INDIVIDUALS JUVENILE IDIOPATHIC ARTHRITIS

Little is known about the possible relationship between periodontitis and JIA. Thus, a systematic approach to review the pertinent literature was not possible. Higher plaque and gingivitis levels and higher caries prevalence have been described in these individuals (Siamopoulou et al. 1989; Welbury et al. 2003). Periodontitis outcomes, such as probing depth and attachment loss, had not been assessed until our first study was conducted and subsequent studies of Havemose-Poulsen et al. (2006), who evaluated young adults, and Reichert et al (2006), who evaluated adolescents. With respect to gingivitis, the first study showed similar conditions between individuals with JIA and controls. In the second study, JIA patients showed increased levels of plaque measured by the approximal plaque index but no differences were observed for the sulcular bleeding index. Periodontitis outcomes were about the same in both studies except for the mean percentage of sites with AL > 3.5 mm which was higher in teenagers JIA patients (Reichert et al. 2006).

POSSIBLE COMMON MECHANISMS

In RA/JIA and periodontitis, there are a number of possible pathways of similar tissue destructive mechanisms, including characteristics of the innate immune response. Neutrophils play an important role in tissue destruction and an aberrant neutrophil activity has been described in JIA, adult RA and periodontitis (Figueredo et al. 1999a; Sikora et al. 1994). Another common pathogenic link affecting periodontitis and JIA, suggested by Mercado et al. (2001), is the monocytic hypersecretory state. Challenges to the monocytic/lymphocytic axis may induce the excessive proinflammatory cytokine secretion, such as IL-1β and IL-18, among others, and consequently stimulation of degrading enzymes and tissue destruction.

Neutrophil activity

Neutrophils have a central role in the process of tissue destruction of RA, JIA and periodontitis as well (Figueredo et al. 2004; Foell et al. 2004; Kantarci et al. 2003;

Pillinger & Abramson 1995). In arthritis, one of the most marked histopathological characteristics is the articular tissues invasion by leukocytes. Neutrophils are attracted by a series of signals and are found mainly at the synovial fluid and at the junction of the inflammatory pannus with cartilage. This finding suggests that neutrophils are responsible for part of articular tissues destruction, specially cartilage (Edwards & Hallett 1997; Foell et al. 2004; Liu & Pope 2004; Pillinger & Abramson 1995). In periodontitis, the neutrophil role has been described as protective and destructive. Individuals with deficiencies in neutrophil quantity and function, such as in cyclic neutropenia and Chediak-Higashi syndrome, are more predisposed to periodontal diseases. On the other hand, products derived from neutrophil activity in excess contribute to periodontal tissue destruction (Buchmann et al. 2002a; Figueredo et al. 2004; Van Dyke & Serhan 2003). Both in gingivitis and periodontitis the number of neutrophils increases in gingival tissues and in the GCF (Wilton et al. 1977; Zappa et al. 1992; Zappa et al. 1991). In this context, neutrophils are recruited to eliminate bacteria and their products through mechanisms of adhesion, chemotaxis, phagocytosis and microbiocidal activity (Kantarci et al. 2003). These cells accumulate mainly in the adjacencies of junctional and sulcular epithelia and are also found in the GCF while in the connective tissue they represent only 3% of the inflammatory infiltrate (Lo et al. 1999; Zappa 1995). Gustafsson et al. (1994) showed that the number of neutrophils in sites with gingivitis and periodontitis seems to be similar, suggesting that might be the hyperactivity of these cells, rather than their number, a decisive factor in the destructive process in periodontitis. A hyperactivity or excessive release of neutrophilic proteases and oxygen free radicals has been observed in RA, JIA and periodontitis (Edwards & Hallett 1997; Figueredo et al. 2004; Sikora et al. 1994).

Elastase is one of the most destrucive proteases involved in chronic inflammatory tissue destructive diseases (Momohara et al. 1997). This enzyme is a neutral serine protease stored in the primary or azurophilic netrophil granules which degrades, besides elastin, collagen and proteoglycans, among other components of the extracellualr matrix (Janoff 1985). When released in the tissue, elastase is normally almost immediately counteracted by two inhibitors, α-1-antitripsin (A1AT) and α-2-macroglobulin (A2MG). If there is an excessive release and/or an inadequate inhibition of elastase, tissue destruction can occur

(Figueredo & Gustafsson 1998; Janoff 1985). Elevated levels of free and complexed to A1AT elastase have been observed in GCF of periodontitis patients (Buchmann et al. 2002a; Buchmann et al. 2002b; Figueredo et al. 2005; Gustafsson et al. 1992). A high elastase activity has also been found in the synovial fluid and plasma of individuals with RA (Bazzichi et al. 2002; Beyeler et al. 2000; Momohara et al. 1997). Levine et al. (1993) reported elevated levels of elastase in the synovial fluid of patients with JIA.

Matrix metalloproteinase-8 (MMP-8) is an interstitial collagenase involved in normal tissue remodelling as well as collagen degradation. MMP-8 is upregulated in pathologic inflammatory conditions, such as atherosclerosis, emphysema, RA and periodontitis (Sorsa et al. 2004; Van Lint & Libert 2006). It is mainly produced by neutrophils being stored in their secretory granula and released from the cells to the inflammatory lesion during migration (Bentwood & Henson 1980). Therefore, it can be regarded as a surrogate marker of the number of neutrophils in the area and as a marker of the severity of inflammation. Besides, this enzyme can be also produced by other cell types, such as epithelial cells and gingival fibroblasts, in response to pro-inflammatory mediators like TNF-α and IL-1β (Kiili et al. 2002; Sorsa et al. 2004). MMP-8 is released in a latent form and requires extracellular activation. Its inhibition is performed by tissue inhibitor of metalloproteinase (TIMP) and A2MG (Romanelli et al. 1999).

Neutrophil activity can also be represented by the expression and activity of MMP-8 in gingival tissues and GCF. It is probably the main type of collagenase in GCF from patients with periodontitis (Ingman et al. 1996; Tervahartiala et al. 2000) and it has been suggested to be suitable for monitoring periodontal conditions (Chen et al. 2000; Sorsa et al. 1999). Higher levels of MMP-8 are found in GCF of sites with tissue destruction compared to sites with gingivitis and healthy ones (Ingman et al. 1996; Mantyla et al. 2003). Some data show that it is the activity rather than the amount of MMP-8 that differs between sites with periodontitis and those with gingivitis (Romanelli et al.1999). In RA, MMP-8 levels are higher in synovial fluid and serum than in patients with osteoarthritis or controls (Tchetverikov et al. 2004). Rajasekhar et al. (2004) demonstrated that immune complexes and a combination of IL-18 and PMA elicit MMP-8 secretion from neutrophils from RA

patients and thus suggest that serum MMP-8 might an indicator of acute inflammatory activity.

Pro-inflammatory cytokines

IL-1β and TNF-α are proinflammatory cytokines of the innate immune system essential in the pathogenesis of RA, JIA and periodontitis (Choy & Panayi 2001; Graves & Cochran 2003; Seymour & Gemmell 2001). These cytokines have a similar biological effect and are important regulators of other active mediators in periodontal and rheumatological inflammations, like PGE_2. In addition, they stimulate MMPs and other proteases released by mesenchymal cells and therefore are related to the processes of tissue destruction (Abbas et al. 2000). Despite their similar effects, TNF-α is less potent than IL-1β rengarding the capacity of phagocyte activation (Kurlander et al. 1989). IL-18 is a member of IL-1 family and has effects in the host response against infeccions and in tissue-destructive inflammations through the induction of IFN-γ IL-1β and TNF-α (Dinarello & Fantuzzi 2003b; Kashiwamura et al. 2002).

IL-1β is a 17 kD protein produced not only by monocytes and macrophages but also by neutrophils, endothelial and epithelial cells (Abbas et al. 2000; Seymour et al. 2001) after stimulation by bacterial products, such as lypopolissacharide, and other cytokines, e. g. TNF and IL-18 (Abbas et al. 2000; Dinarello 1996; Jablonska et al. 2001). The biological activities of IL-1β are multiple and contribute to systemic inflammation, through induction of acute phase proteins, and to local inflamation. Included in these activities are the induction of adhesion molecules and chemokines and the enhancement of neutrophil degranulation (Brandolini et al. 1997). IL-1β also affects fibroblasts, activating collagenase and PGE_2 production and increases osteoclastic activity (Dinarello 1996; Domeij et al. 2005; Kawai et al. 2006). In an animal model of collagen induced arthtiris, the injection of IL-1β in the joints induced destruction of articular tissues. This was couteracted by the administration of monoclonal antibodies against IL-1β (Joosten et al. 1996). Elevated levels of this cytokine were observed in synovial fluid of RA (Lettesjo et al. 1998; Rooney et al. 1990) and JIA patients (Madson et al. 1994). Systemically, in serum, IL-1β is elevated in both conditions according to some studies (Altomonte et al.

1992; Madson et al. 1994; Yilmaz et al. 2001), while in others not (De Benedetti et al. 1995). Havemose-Poulsen et al. (2005) found no difference in plasma levels of IL-1β comparing individuals with localized aggressive periodontitis, generalized aggressive periodontitis, RA, JIA and healthy controls. In this same study, patients with aggressive periodontitis, juvenile idiopathic arthritis or rheumatoid arthritis shared similar blood cytokine profiles (higher IL-10 levels in plasma and higher TNF-α in unstimulated cultures) distinguishing them from individuals free of disease.

Experimental studies show that IL-1β is highly involved in the mechanisms of periodontal tissue destruction. Its application accelerates inflammation and bone loss in rats and its blockade significantly reduces tissue desctruction in experimental (Delima et al. 2002; Koide et al. 1995). Different cell types express IL-β in the periodontal tissues such as monocytes/macrophages, neutrophils, fibroblasts, B cells, epithelial cells (Jandinski et al. 1991; Lo et al. 1999). Experimentaly induced arthritis in rats significantly increased the levels of IL-1β and MPP-8 in the periodontal tissues in comparison to control rats (Ramamurthy et al. 2005). IL-1β is elevated in GCF of site with periodontitis compared to healthy ones (Figueredo et al. 1999b; Hou et al. 2003; Ishihara et al. 1997). In a longitudinal study, sites considered to be active showed higher amounts of IL-1β in GCF compared to inactive sites (Gamonal et al. 2000). When analysed in serum/plasma of periodontitis patients, IL-1β levels are low or undetectable in some studies (Albandar et al. 2002; Mengel et al. 2002) whilst a tendency towards higher levels than controls was observed by Figueredo et al. (2000).

IL-18 is a member of the IL-1 cytokine family. Pro-IL-18 is cleaved by caspase-1 (IL-1β-converting enzyme) to yield biologically active 18-kDa IL-18 (Dinarello & Fantuzzi 2003; Kashiwamura et al. 2002). Upregulation of IL-18 has been described in a range of different chronic diseases, including type I diabetes, lupus, and Crohn's disease (McInnes et al. 2000). This cytokine is expressed in various types of cells, including macrophages, keratinocytes, intestinal epitherial cells, osteoblastic cells, chondrocytes, and adrenal cortex cells. IL-18 promotes IFN-γ production and Th1 helper T-cell development, synergistically with IL-12. However, IL-18 itself shows capabilities to induce IL-4, IL-5,

IL-10, and IL-13 from T and natural killer cells (Kashiwamura et al. 2002). Additionally, IL-18 has an important role on developing innate immune responses IL-18 has been implicated in the activation of neutrophils (Leung et al. 2001) and modulation of IL-1β production (Jablonska et al. 2001). It also induces PGE_2 production from activated macrophages (Kashiwamura et al. 2002). In RA and JIA patients, serum levels of IL-18 are higher than those observed in healthy controls (Bresnihan et al. 2005; Maeno et al. 2002; Yamamura et al. 2001). An exacerbated expression of IL-18 has been observed in macrophages and synoviocytes from articular tissues of RA patients (Gracie et al. 1999; Joosten et al. 2003; Yamamura et al. 2001). The expression of IL-18 is highly related to the expression of IL-1β and TNF-α in the synovial tissues, suggesting an interdependent regulation among these cytokines in RA (Joosten et al. 2003). Scola et al. (2002) reported a high expression of IL-18 in the synovial tissues of a group of JIA patients from all subtypes of this condition.

Oral epithelial cells, which are the primary host-pathogen interface, have been reported to produce IL-18 in response to lypopolysaccharide stimulation (Rouabhia et al. 2002; Sugawara et al. 2001). There was a lack of further information about the role of IL-18 in periodontitis until the publications of Johnson & Serio (2005) and Orozco et al. (2006). These authors showed higher concentrations of IL-18 in gingival biopsies and GCF mainly from sites with periodontitis.

EFFECT OF MEDICATIONS

Once the great majority of RA and JIA patients are under pharmacological treatment, the effect of these medications on inflammatory markers and periodontal tissues should be considered.

NSAIDs acts through inhibition of cyclooxygenase (Vane & Botting 1998). Other modes of action include inhibition of IL-1 and IL-6 production by mononuclear cells, as well as having multiple effects on other cellular responses, such as polymorphonuclear cell

degranulation, calcium influx and phosphorylation of intracellular proteins (Bondeson 1996). Animal models and clinical trials support the hypothesis that the inhibition of arachidonic acid metabolites through NSAIDs is able to retard the progression of periodontitis (Paquette & Williams 2000).

Glucocorticoids affect virtually every cellular and humoral mechanism involved in inflammation, including neutrophils and cytokines. Its mechanism of action is complex and is related to regulation of genes transcription, inhibiting proinflammatory and chemotatic cytokines such as IL-1, TNF-α and IL-8 (Boss et al. 1999; Lee et al. 1988). Patients taking glucocorticoids for other reasons show less gingival inflammation but similar clinical attachment levels than controls (Markitziu et al. 1990; Saether et al. 1998).

With respect to cyclosporine, its effect as an inductor of gingival hyperplasia is well recognized, however results from studies evaluating its effects on the periodontium of insertion are conflicting. Ferrets treated with cyclosporine showed more attachment loss (Fischer & Klinge 1994). On the other hand, this immunossupressor was not able to aggravate alveolar bone loss in rats (Goncalves et al. 2003). Additionally, clinical studies were not able to observe an effect of cyclosporine on periodontitis parameters (Oettinger-Barak et al. 2001; Saether et al. 1998).

MTX has effects on neutrophils superoxide anion formation and adhesion, on the modulation of cytokine responses at a number of levels and on the promotion of apoptosis of activated lymphocytes (Chan & Cronstein 2002; Genestier et al. 2000; Neurath et al. 1999). This folic acid antagonist acts in the low doses used for JIA and RA probably act as an anti-inflammatory agent (Whittle & Hughes 2004). The influence of MTX on the periodontal conditions remains unknown.

HYPOTHESIS

Considering that in adults there is the possibility that RA might be a modifier factor of periodontitis and considering the limitation of available information about the periodontal

conditions of JIA patients the study of the periodontal status, through cross-sectional and especially longitudinal approaches, in this group are needed. In addition, possible common underlying mechanisms between both diseases and the possible interference of medications in these processes would contribute to better understand the relationships of RA/JIA and periodontitis.

This thesis was therefore conducted based on the hypothesis that adolescents with JIA might have a different periodontal condition due to common altered inflammatory and destructive mechanisms. Additionally, JIA activity and anti-rheumatic medications might influence these mechanisms.

AIMS OF THE THESIS

GENERAL AIM

The general aim of this thesis was to relate periodontal conditions, clinical status as well as degree of inflammation assessed as amounts of inflammatory markers in GCF, to rheumatic disease activity and anti-rheumatic medication in adolescents with JIA.

SPECIFIC AIMS

- To compare the periodontal conditions in a group of adolescents with JIA with those in a healthy group; to relate their periodontal condition to their rheumatological status.

- To investigate the possible effect of JIA aberrant inflammatory response on serum and GCF markers of inflammation; to evaluate the subgingival microbiota of JIA and control groups.

- To monitor changes in periodontal inflammation in individuals with JIA for 2 years after continuous rheumatological treatment;

- To evaluate in a group of individuals with RA under rheumatological treatment the effect of anti-rheumatic medication on markers of periodontal inflammation in GCF.

MATERIAL AND METHODS

This section is a brief summary of the material and methods employed. Additional and detailed information can be found in each individual paper.

ETHICAL CONSIDERATIONS

Studies I, II and III were approved by the Ethics Committee of Pedro Ernesto University Hospital, Rio de Janeiro State University, Rio de Janeiro, Brazil and Huddinge Hospital, Karolinska Institutet, Huddinge, Sweden. Study IV was approved by the Ethics Committee of Brazilian Lutheran University, Canoas, Brazil and Huddinge Hospital, Karolinska Institutet, Huddinge. All studies were conducted in accordance with the Helsinki Declaration and all volunteers and parents/guardians gave written informed consent to participate.

EXPOSURE DEFINITION

In studies I, II and III the exposure was defined as the presence of JIA diagnosed by one and the same pediatric rheumatologist according to the ILAR classification of 1997 (Petty et al. 1998). The rheumatological clinical evaluation consisted of the recordings of the number of joints with edema, the number of joints with pain, the number of joints with limitation on movement (LOM) and the total number of joints affected. The physician's global assessment (PGA) was noted on a visual analogue scale ranging from 0 to 10 (inactive to severe) (Foster et al. 2003). Information regarding type, duration of disease and medications in use was collected.

In study IV the exposure was defined as the presence of RA diagnosed by one and the same rheumatologist based on the criteria of the American College of Rheumatology of 1987 (Arnett et al. 1988).

PARTICIPANTS SELECTION

Exposed groups

Based on the exposure definitions described above, the exposed group was constituted of 38 adolescents diagnosed with JIA in attendance at the Pediatric Rheumatology Clinic of NESA, Rio de Janeiro State University, Rio de Janeiro, Brazil (Studies I, II and III) and 17 adults with RA from the Rheumatology Clinic of the Brazilian Lutheran University Hospital, Canoas, Brazil (Study IV).

In Study I, the exposed group comprised 32 adolescents with JIA (mean age 15.9±2.7 years, 22/32 females) and in Study II, six more adolescents were added (mean age 15.6±2.7 years, 25/38females). Individuals with other conditions, like diabetes, were not included. These individuals were selected consecutively during the period of September 2001 and June 2002. In Study III, 18 of these adolescents were reexamined after 2 years (mean age 17.3±2.6 years, 9/18 females).

To be included in the exposed group of Study IV the individuals with RA should not have any other systemic disease, should not have been submitted to periodontal treatment previously. The mean age of this group was 49.5±10.6 years and 15 out of 17 participants were women. The mean duration of RA was 12.1 (± 9.9) years. The median erythrocyte sedimentation rate of these individuals, in mm/h, was 30.5, with quartiles 4 and 91.

Non-exposed groups (controls)

To comprise the non-exposed group, 29 adolescents were selected consecutively from the General Pediatric Clinic of NESA, Rio de Janeiro State University, Rio de Janeiro, Brazil (Studies I, II and III) and 17 systemically healthy adults from the Periodontology Clinic of the Federal University of Rio Grande do Sul and Brazilian Lutheran University, Brazil (Study IV).

In Study I, 24 controls were selected (mean age 14.7+2.3 years, 12/24 females) and for Study II, five adolescents were added (mean age 14.7+2.3 years, 16/29 females) if they showed no signs of ongoing infections or inflammatory diseases and after their physician

had ensured they had no disease and were not taking any medication. In Study III, 14 of these adolescents were reexamined after 2 years (mean age 16.6±1.5 years, 5/14 females).

The non-exposed group of Study IV was matched for age, gender, periodontal status and tobacco use. To be included in this group the participants should not have had any other systemic disease, should not have been submitted to periodontal treatment previously. The mean age of this group was 48.6±11.2 years and 15 out of 17 subjects were women.

QUESTIONNAIRES

Participants of Studies I, II and III answered the Brazilian version of the Children Health Assessment Questionnaire (CHAQ) ranging from 0 to 3 (Machado et al. 2001) and registered on a visual analogue scale ranging from 0 to 10 how they felt regarding JIA, the patient's global evaluation (Singh et al. 1994). They were also asked about smoking habits.

In Study IV, the participants answered a questionnaire about their personal, demographic, rheumatological and periodontal data.

PERIODONTAL EVALUATION

For the four studies, the periodontal examination consisted of the recordings of visible plaque, marginal bleeding, probing depth, clinical attachment level. In addition, the number of present teeth and the percentual of sites with bleeding on probing were registered in Study IV.

A participant was considered to have periodontal attachment loss (AL) when an AL of 2 mm or more was detected in proximal sites by probing (Studies I, II and III).

SAMPLES COLLECTION

Blood

Blood samples were taken by venipuncture from all participants of Studies I, II and III. Part of it was used for erythrocyte sedimentation rate (ESR) determination and the rest was centrifuged. Sera aliquots were stored frozen at –70°C pending analysis.

Gingival crevicular fluid (GCF)

After the periodontal examination, GCF was collected through the method of intracrevicular washings modified from (Salonen & Paunio 1991). Four to 6 non-adjacent sites with the deepest probing depths were sampled in each of all participants (Studies I, II, II and IV). Each person's samples were pooled together and diluted in phosphate buffer solution up to 1 ml. The samples were centrifuged and the supernatant frozen at -70^0C until analysis.

Subgingival plaque

In Study II, subgingival plaque was collected in the same sites, after GCF sampling, by placing sterile paper points inside the crevice for 30 seconds. The paper points were subsequently placed, pooled together for each subject, in sterile transport vials and sent to a laboratory for bacterial DNA-probe analysis (Göteborg University, Sweden).

ELASTASE ACTIVITY ASSAY

Measurement of the elastase activity was performed in Studies II and IV as previously described by Figueredo et al. (1999). Briefly, 100 μl of sample was mixed with 67 μl of the granulocyte elastase substrate s-2484 (l-pyroglutamyl-l-prolyl-l-valine-p-nitroaniline, mw 445.5 da., Haemochrom Diagnostica AB, Mölndal, Sweden) on 96-well microtitre plates. The mixture was incubated at +37°c and the absorbency at 405 nm was read after 2 hours in a spectrophotometer (Millenia Kinetic Analyzer, Diagnostic Product Corporation, Los Angeles, CA, USA). The elastase activity was expressed as milliabsorbances (mabs). To inhibit the elastase activity, 10 μl of α-1-antitrypsin (A1AT) 0.1% was added to 90 μl of sample and incubated during agitation for 15 min at room temperature. After inhibition, the samples were tested against elastase activity, as described above. The elastase activity inhibited by A1AT was regarded as free elastase and the remaining activity as elastase inhibited by α-2-macroglobulin (A2MG).

IL-1β, IL-18 AND MMP-8 ASSAYS

In serum, IL-1β and IL-18 were measured with an enzyme-linked immunosorbent assay (ELISA) by commercially available kits (IL-1β Quantikine HS, R&D Systems, Minneapolis, MN and IL-18, MBL, Nagoya, Japan). Both analyses followed the respective manufacturers instructions and IL-1β and IL-18 levels were expressed as pg/mL.

In Studies II, III and IV, IL-1β was also measured in GCF by ELISA. Shortly, a monoclonal antibody against IL1-β (MAB 601, R & D Systems, Minneapolis, MN, USA) diluted 125 times in carbonate buffer was coated onto microtitre plates overnight at 4°C. After washing, the plates were blocked with 1% human serum albumin for 1 hour at room temperature, washed again and a standard curve (2 pg/mL to 200 pg/mL) and undiluted samples (100 μl) were added. The plates were incubated at 37^0C, washed and the detection antibody (BAF 201, R & D Systems, Minneapolis, MN, USA) diluted 250 times was incubated as described above. After washing, streptavidin, diluted 200 times, was added to the plates and incubated further at 37^0C. The plates were once again washed and the undiluted substrate added (TMB, Sigma Chemical, St. Louis, MO, USA). The reaction was stopped with 1M H_2SO_4 after 15 minutes and the absorbency read at 450 nm in a spectrophotometer. IL-1β in GCF was expressed as amounts per subject (pg).

IL-18 (Studies III and IV) and MMP-8 (Study III) were measured in GCF by commercially available ELISA kits according to the manufacturers instructions (IL-18, MBL, Nagoya, Japan and Human MMP-8 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA). Total amounts of IL-18 were expressed as pg and of MMP-8 as ng.

MICROBIOLOGICAL PROCESSING

In Study II, subgingival plaque was analyzed by the checkerboard DNA-DNA hybridization method. Digoxigenin-labelled whole genomic DNA probes were applied in order to detect 12 bacteria: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythensis*, *Actinobacillus actinomycetemcomitans*,

Fusobacterium nucleatum, Treponema denticola, *Peptostreptococcus micros*, *Campylobacter rectus*, *Eikenella corrodens*, *Selenomonas noxia* and *Streptococcus intermedia*. Standardized procedures of this method were used (Papapanou et al. 1997). The hybrids formed between the bacterial DNA and the probes are detected by applying an antidigoxin antibody conjugated with alkaline phosphatase and incubated with a chemiluminescent substrate. Evaluation of the chemiluminescent signal is performed by comparing the signals obtained with those of pooled standard samples containing 10^6 or 10^5 of each of the 12 species. The chemiluminiscent units obtained are transformed into a scale from 0 to 5, where 0 indicates no signal; 1, a signal weaker than that of the low standard (10^5 bacteria); 2, a signal equal to the low standard; 3, a signal higher than that of the low standard but lower than that of the high standard (10^6 bacteria); 4, a signal equal to that of the high standard; and 5, a signal higher than that of the high standard. Thus, 6 scores (0-5) are used for each species. Frequency of positive sites and frequency of sites with $\geq 10^6$ bacterial cells in each sample were determined.

LABORATORY MARKERS OF JIA ACTIVITY

The ESR (Studies I, II, III and IV) and C-reactive protein (CRP) (Studies I and II) were analyzed as laboratory markers of JIA disease activity.

The ESR, in mm/h, was determined by the Westergren method in the first hour. The levels of CRP expressed in mg/L were analyzed with a commercial kit (N High sensitive CRP, Dade Behring AG, Marburg, Germany) in accordance to the manufacturer's instructions using a nephelometer analyzer (Behring AG Diagnostica, Marburg, Germany).

STATISTICAL ANALYSIS

In general, the unit of analysis was established as the individual and α was set at 0.05. Normally distributed data was presented by means of means and standard deviations and non-normally distributed, medians and percentiles 25/75. *Statistica 6.0* and *7.0* softwares were used to analyze the data.

In Study I, the one-tailed Fisher's exact and Mann–Whitney tests were used when appropriate.

In Study II, Mann-Whitney, Kruskal-Wallis, Dunn's and Fisher exact tests were applied as well as Spearman's correlation coefficient calculated as indicated in the text/tables.

In Study III, Wilcoxon and Mann-Whitney tests as well as Spearman correlation coefficients were used.

In Study IV, frequency descriptions, correlations and Mann Whitney test were calculated.

RESULTS

STUDIES I AND II

Periodontal conditions in patients with juvenile idiopathic arthritis

In this section, the clinical, inflammatory markers and microbiological findings of all participants of Studies I and II are presented.

The frequency of individuals with JIA having at least one site with proximal AL≥ 2 mm was 23.7% (9/38) versus 3.4% (1/29) in the control group (Fisher's exact test, p=0.0348). This criterion was used to divide the JIA group in those having AL (JIA AL) and those not having it (JIA NoAL). Table 3 summarizes the periodontal findings regarding the clinical and GCF inflammatory parameters in both groups. Systemic markers of inflammation are also shown.

Clinically, supragingival parameters, such as visible plaque and marginal bleeding, were similar in JIA and control groups. This same pattern was observed for the subgroups of the JIA group regarding AL. The mean percentages of sites with PD ≥4 mm and of sites with proximal AL>2mm were significantly higher in the JIA group than in the CTR group. In the JIA AL subgroup the mean number of sites affected by AL ≥ 2 mm was 3.7 (± 2.2) and the mean number of teeth affected was 3.0 (± 1.6). The only adolescent with AL in the control group showed 45% and 30% of sites with visible plaque and marginal bleeding, respectively. The mean percentages of sites with PD ≥4 mm was 8.3% and of sites with proximal AL>2 mm, 1.2%. None of the participants of both groups reported to smoke.

Regarding the clinical rheumatological status, JIA individuals showing AL were mostly from the systemic and arthritis related to enthesitis subtypes of JIA. The duration of disease was similar between those with AL (53±44 months) and those without it (57±42 months). However, the JIA AL subgroup tended to be more affected by the arthritic disease as shown by a significant higher mean number of joints with LOM and higher median of patient's global assessment. Total numbers of joints affected by pain or edema were also

higher in JIA AL subgroup compared to JIA NoAL, although not statistically different. Of all JIA patients, 60.5% (23/38) were regarded as having active JIA with and ESR> 20 mm accompanied by clinical signs of arthritits. Of 9 JIA patients with AL, 8 (89%) were undergoing treatment with a DMARD (methotrexate) or a combination of NSAID (nimesulide or naproxen or indomethacin) + DMARD (methotrexate) + immunossupressant (glucocorticoids or cyclosporine). In JIA No AL, this proportion was 16/29 (55%). No treatment-related significant differences were found in the periodontal clinical variables and inflammatory markers studied.

With respect to the inflammatory markers, except for the E+A2MG complex, there were no significant differences in the GCF levels between JIA and controls. The percentage of free elastase in GCF (30% versus 13%) and CRP (4.6 mg/L versus 0.8 mg/L) tended to be higher in JIA with AL, although these values did not differ statistically. Systemically, ESR, CRP, IL-1β and IL-18 were significantly elevated in patients with JIA compared to controls. JIA patients with AL presented significantly higher levels of IL-18 in serum. This was the only inflammatory marker significantly increased in active JIA patients with AL compared to active JIA without AL, inactive JIA with or without AL Significant correlations were found between serum IL-18 and the following variables: IL-1β, ESR and CRP and also between serum IL-18 with the periodontal variables, AL and PD.

Microbiologically, controls showed a higher frequency of positive sites for *Fusobacterium nucleatum*, *Peptostreptococcus micros* and *Campylobacter rectus*. However, no differences between the groups were observed when the frequency of sites with $\geq 10^6$ bacterial cells were compared. Although we did not observe significant differences between JIA NoAL and JIA AL patients with respect to the subgingival microbiota, the frequency of subjects positive to *Porphyromonas gingivalis* was 44.4% in the JIA AL subgroup versus 17.9% in the JIA NoAL subgroup (Table 4).

STUDY III

Changes in periodontal and rheumatological conditions after 2 years in patients with juvenile idiopathic arthritis

The periodontal and rheumatological parameters of the JIA and control groups at the baseline and the 2-year examinations are shown in Table 5. The systemic inflammatory status, measured by the ESR, and the clinical rheumatological parameters significantly decreased over the 2-year period. The prevalence of subjects with incipient AL was 22% (4/18) in the JIA group *versus* 14% (2/14) in the control group. Three of these subjects with incipient attachment loss at the 2-year examination were considered to be in an active episode of JIA. Plaque levels significantly decreased from baseline in both groups. For marginal bleeding levels, there was no difference between the baseline and the second examination. A significant increase was apparent in the percentage of sites with PD>4 mm in both groups. This was not observed in the percentage of sites with AL>2 mm. However, at the 2-year examination, no significant differences were observed between groups for the periodontal clinical parameters.

In GCF, the total amounts of IL-1β decreased between baseline and the follow up in both groups. The decrease was significantly more pronounced in the JIA group (Wilcoxon test, $p=0.027$). Table 5 also depicts the total amounts of MMP-8 and IL-18 in the GCF measured at the 2-year examination. When JIA and the control groups were compared, no significant difference was found for these markers. The JIA patients with active disease had less IL-1β, MMP-8, and IL-18 in GCF compared to patients with inactive disease. There were no differences in periodontal status between these groups.

Significant correlations were found among MMP-8 and IL-1β levels ($rs = 0.39$; $p<0.05$) and among IL-1β and IL-18 ($rs = 0.44$; $p<0.05$) in GCF. Additionally, significant correlations were found between the percentage of sites with AL$\geq$2mm and ESR ($rs=0.504$, $p<0.05$) and total amount of IL-1β in GCF ($rs=0.513$, $p<0.05$) in the JIA group. These correlations did not hold significant for the control group.

STUDY IV

Decreased IL-1β and elastase in the GCF of individuals with rheumatoid arthritis

The number of present teeth was lower in RA group, although not statistically different from the controls. In the other clinical periodontal parameters analyzed, visible plaque, marginal bleeding, bleeding on probing and extensions of cathegorized PD and AL, there were no significant differences between these groups.

With regards to the medications in use by the RA individuals and their smoking status, 88,2% of the subjects used prednisone, 76,5% used methotrexate, 76,5% used NSAID and 23,5% used sulfasalazine. In relation to smoking habits, 3 pairs were regular smokers (17,6%) and 4 reported to be former smokers (23,5%). The mean duration of RA was 12.1 ($\pm$ 9.9) years. The median ESR rate, in mm/h, was 30.5, with quartiles 4 and 91.

The results of the GCF biomarkers of periodontal inflammation are described in Table 6. Total amounts of IL-1β (Mann-Whitney test, $p=0.008$) and total elastase (Mann-Whitney test, $p=0.003$) were significantly lower in the RA group compared to CTR group. IL-1β and total elastase were significantly and highly correlated in the RA group ($r=0.883$, $p<0.001$). This correlation could not be observed in the control group.

Figures 2 and 3 show the combined data of GCF IL-1β from Studies II and IV and GCF IL-18 from Studies III and IV, respectively. Higher amounts of IL-1β in GCF of individuals with JIA (median 66.6, quartiles 36.5/83.5) and adult controls (median 58.3, quartiles 30.5/83.5) from Study IV and lower amounts for adolescent controls of Study II (median 41.6, quartiles 18.5/64.0) and RA subjects (median 18.6, quartiles 12.7/41.4) were observed. With regards to IL-18, similar medians were found for the four groups with higher 75 percentiles for JIA and adult control groups.

Table 3. Periodontal variables (mean (SD)), GCF and serum inflammatory markers (median (25^{th}-75^{th}percentiles)) in JIA and control groups and JIA NoAL and JIA AL subgroups.

	CLINICAL VARIABLES				**GCF MARKERS**					**SYSTEMIC MARKERS**			
	Plaque (%)	**Bleeding (%)**	**PD**	**AL**	**Total E (mAbs)**	**E+α2MG (mAbs)**	**Free Elastase (mAbs)**	**%Free Elastase**	**IL-1β^ (pg)**	**ERS (mm/h)**	**CRP (mg/L)**	**IL-1β (pg/mL)**	**IL-18 (pg/mL)**
JIA n=38	55 (22)	31 (15)	3.0 (3.4)	0.7 (1.3)	25 (13-47)	20 (7-36)	3 (0-9)	19 (4-60)	66.6 (36.5-83.5) n=19	41 (18-48)	1.0 (0.3-12.5)	1.2 (1.0-1.4)	679.5 (556.4-865.3)
Control n=29	47 (18)	33 (16)	0.4 (1.6)	0.04 (0.2)	38 (18-72)	27 (18-46)	4 (0-36)	12 (8-34)	41.6 (18.5-64) n=23	13 (6-20)	0.4 (0.2-1.3)	0.3 (0.0-0.4)	363.6 (330.5-397.0)
p_1	n. s.	n. s.	0.018	<0.001	n.s.	0.035	n.s.	n.s.	n.s.	<0.001	0.014	<0.001	<0.001
JIA NoAL n=29	54 (22)	31 (16)	1.4 (2.0)	0 (0)	26 (15-47)	19 (8-36)	3 (1-35)	13 (4-70)	71.1 (36.5-91.3) n=15	41 (18-47)	0.8 (0.3-10.2)	1.0 (0.9-1.5)	648.0 (512.7-736.9)
JIA AL n=9	58 (20)	31 (13)	5.4 (5.0)	2.8 (1.1)	16 (10-38)	20 (7-29)	4 (3-14)	30 (12-33)	39.1 (26.7-53.0) n=4	43 (18-51)	4.6 (0.9-28.5)	1.3 (1.2-1.4)	2230.6 (821.4-10503.4)
p_2	n.s.	n.s.	0.014	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001

JIA NoAL: absence of attachment loss. JIA AL: at least 1 proximal site attachment loss≥2mm.
Total E: total elastase, E+α2MG: elastase + α-2-macroglobulin, ERS: erythrocyte sedimentation rate, CRP: C-reactive protein.
PD: mean % sites with probing depth ≥ 4mm (s.d.). AL: mean % sites with proximal attachment loss ≥2 mm (s.d.).
n.s.: not significant, Mann-Whitney test, p≥0.05. p_1 : value JIA vs. control, p_2 : value NoAL vs. AL. ^sample size indicated(n).

Table 4. Frequencies of positive sites (A) and of sites with $\geq 10^6$ bacterial cells in each sample (B) of microorganisms in subgingival plaque of JIA and controls and JIA AL and NoAL patients.

	Control		**JIA**					
	All		**All**		**NoAL**		**AL**	
	n=29		**n=38**		**n=29**		**n=9**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Porphyromonas gingivalis	42.8	3.5	24.3	0	17.9	0	44.4	0
Prevotella intermedia	89.3	10.7	91.6	5.6	96.3	3.7	77.8	11.1
Prevotella nigrescens	96.4	10.7	94.4	2.8	96.3	3.7	88.9	0
Tannerella forsythensis	82.1	0	80.6	0	81.5	0	77.8	0
Actinobacillus actinomycetemcomitans	57.1	0	55.9	0	65.4	0	33.3	0
Fusobacterium nucleatum	75*	0	47.2	0	51.9	0	33.3	0
Treponema denticola	35.7	0	27	0	28.6	0	22.2	0
Peptostreptococcus micros	100*	0	82	0	82.1	0	77.8	0
Campylobacter rectus	35.7*	0	13.5	0	17.8	0	0	0
Eikenella corrodens	46.4	0	35.1	0	32.1	0	44.4	0
Selenomonas noxia	17.8	0	10.8	0	10.7	0	11.1	0
Streptococcus intermedius	100*	0	70.3	0	75	0	55.6	0

JIANoAL: absence of attachment loss. JIA AL: at least 1 proximal site attachment loss $\geq$2mm.
*:$p<0.05$, Fisher exact test (two-tailed) for All controls versus All JIA and JIA NoAL versus JIA AL.

Table 5. Rheumatological parameters of JIA patients and ESR (mm/h), mean percentages (standard deviation) of visible plaque, marginal bleeding, sites with probing depth (PD) ≥ 4mm, sites with proximal attachment loss (AL) ≥ 2 mm and medians (percentiles 25/75) of the total amounts of IL-1β (pg), MMP-8 (ng) and IL-18 (pg) in GCF of the JIA and control groups at baseline and 2-year examinations.

	RHEUMATOLOGICAL PARAMETERS								**PERIODONTAL PARAMETERS**						
	ESR	**Swell**	**Pain**	**LOM**	**Total**	**PGA**	**CHAQ**	**PatGA**	**Plaque**	**Bleeding**	**AL>2**	**PS>4**	**IL-1β**	**MMP-8**	**IL-18**
JIA (n=18)															
Baseline	40.9	6.4	7.4	7.3	21	3.2	0.6	3.3	54.6	29.1	1	2.5	66.6		
	(34.4)								(19.8)	(12.1)	(1.3)	(2.9)	(39.4-73.3)		
2 ys	26.2	3.0	3.9	5.4	5.8	2.2	0.6	1.9	39.4	41.6	0.4	8.7	14.6	10.8	41.2
	(26.5)								(17.9)	(25.8)	(0.9)	(12.9)	(10.6-24.3)	(7.4-13.3)	(20.6-103.3)
p	0.055	0.029	0.029	0.017	0.005	0.010	0.624	0.012	0.013	0.147	0.207	0.0026	0.027		
CTR (n=14)															
Baseline	13.3								46.9	33.8	0	0.2	49.8		
	(10.8)								(16.1)	(19.9)		(0.4)	(25.9-73.8)		
2 ys	12.4								34.9	38.9	0.3	5.7	26.5	10.4	37.5
	(11.6)								(18.2)	(13.1)	(0.6)	(7.6)	(19.8-40.7)	(7.5-15.9)	(25.3-62.2)
p	1.0								0.013	0.506		0.005	0.074		
p1	0.015								0.174	0.877	0.015	0.001	0.962		
p2	0.167								0.487	0.866	0.955	0.639	0.099	0.624	0.922

p: time-period comparison, Wilcoxon test, p≤ 0.05. p1: group comparison at baseline, Mann-Whitney test, p1 ≤ 0.05. p2: group comparison at 2ys examination, Mann Whitney test, p2 < 0.05

ESR: erythrocyte sedimentation rate, LOM: limitation on movement, PGA: physician global assessment, CHAQ: Children´s Health Assessment Questionnaire, PatGA: patient global assessment.

Table 6. Means (± standard deviation) of total amounts of IL-1β (pg), IL-18 (pg) and total elastase (abs) in GCF of RA and CTR groups.

	RA n=17	**CTR n=17**	**P**
IL-1β	31.45 ± 34.82	55.73 ± 30.60	0.008
IL-18	50.63 ± 50.77	63.77 ± 67.53	0.786
Elastase	0.15 ± 0.28	0.82 + 0.64	0.003

Significance of the differences between the groups calculated by the Mann-Whitney test.

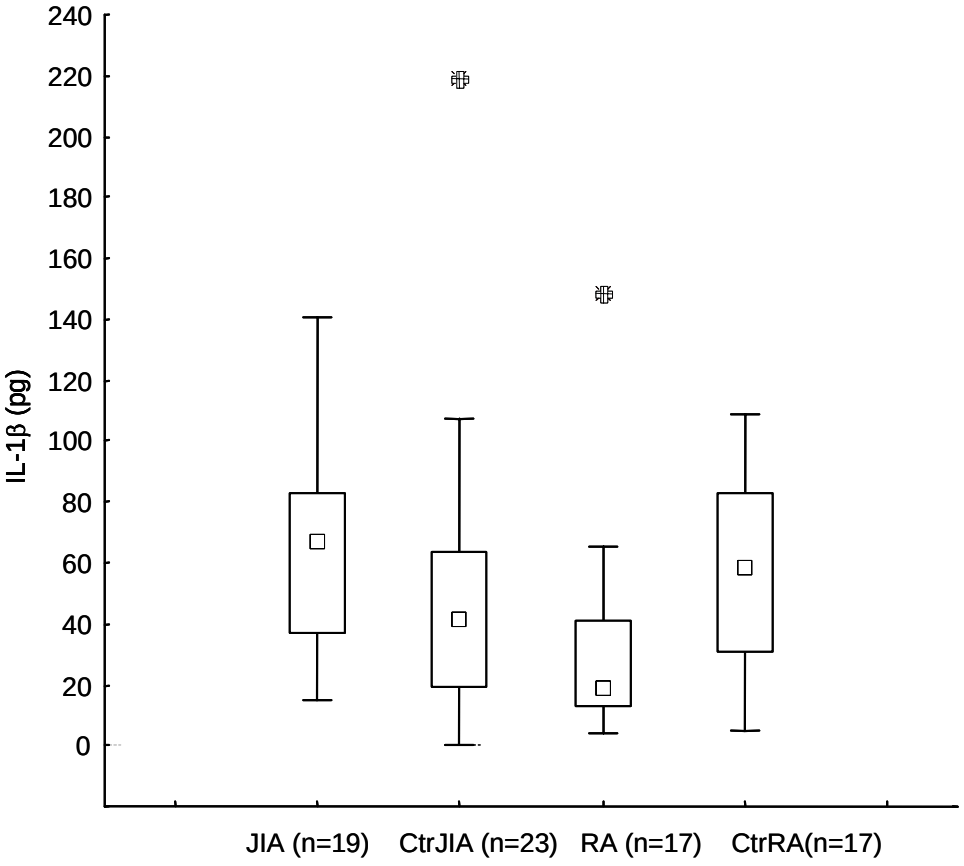

Figure 2. IL-1β (pg) in GCF of groups JIA and control from Study II and groups RA and controls from Study IV.

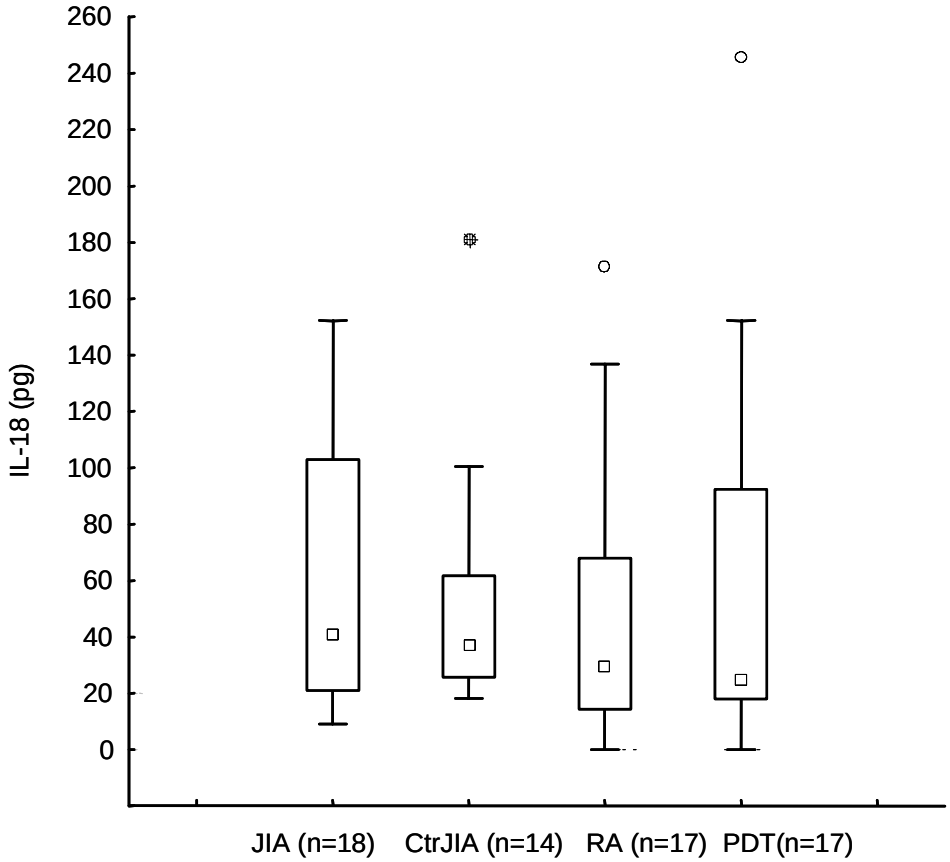

Figure 3. IL-18 (pg) in GCF of groups JIA and control from Study III and groups RA and controls from Study IV.

DISCUSSION

The studies in this thesis show that adolescents with JIA, especially those clinically more affected by the rheumatic disease, have more frequently incipient AL with similar plaque levels, gingivitis levels and subgingival microbiological profile compared to controls. An altered systemic inflammatory state was confirmed in JIA patients since ESR, serum levels of CRP and of two key proinflammatory cytokines, IL-1β and IL-18, were significantly elevated. In GCF of the JIA AL subgroup, a possible reflection of this inflammatory state, represented by a tendency of higher IL-1β and free elastase, could be observed. After 2 years of follow-up, there was a clinical and laboratory rheumatological improvement in JIA patients. Locally, the total amounts of IL-1β in GCF significantly decreased and no differences were observed in periodontitis parameters. Additionally, the anti-inflammatory effect of the medications used for JIA and RA treatment might probably be involved in the decreased levels of IL-1β and elastase detected in GCF of adult RA patients compared to controls matched for periodontal status.

An absence of significant differences in the percentages of sites with plaque and marginal bleeding between JIA and control groups has also been noticed in adults with RA (Gleissner et al. 2001; Sjostrom et al. 1989) and young adults with JIA (Havemose-Poulsen et al. 2006). Kässer et al. (1997) found similar amounts of plaque, but more marginal gingival inflammation in rheumatoid patients than controls. In children, more plaque levels (Reichert et al. 2006) and gingival inflammation has been reported in JIA patients than in controls (Siamopoulou et al. 1989). Factors including use of anti-inflammatory medications, hands and fingers involvement and enrollment in regular dental care programs might be involved in these divergent results.

The prevalence of a proximal AL of 2 mm or more was 23.7% in the JIA group and 3.4% in the controls. Different threshold have been proposed to identify adolescents and young adults with incipient attachment loss: one site with AL $\geq$ 1 mm (Clerehugh et al. 1990), AL $\geq$ 2 mm (Clerehugh et al. 1990; Van der Velden et al. 1989) and AL $\geq$ 3 mm (Albandar et al. 1997; Loe & Brown 1991). Our threshold of AL of 2 mm or

more has been well accepted by others (Jenkins & Papapanou 2001) and the proximal areas were chosen because buccal AL in youngsters may occur due to traumatic tooth brushing (Clerehugh et al.1988). The prevalence of AL in our control group is similar to that reported by others (4.8% by Van der Velden et al. 1989, 3.0% by Clerehugh et al. 1990, 9.7% by Lopez et al. 2001). The findings of early periodontal destruction in JIA individuals observed in the present study are in agreement with observations in adult rheumatoid patients (Kässer et al. 1997; Mercado et al. 2001; Tolo et al.1990). Our findings are also corroborated by the study of Reichert et al. (2006) who found a higher mean percentage of sites with AL > 3.5mm in JIA patients with a mean age of 14.4 years old. Havemouse-Poulsen et al. (2006) observed similar periodontitis outcomes between JIA and controls. However, the JIA patients of that study were 20 to 35 years old and participants of a national dental care program.

To analyze the subgingival microbiota of JIA and control groups we used the checkerboard DNA-DNA hybridization technique which has been widely applied to detect and quantify periodontopathogens (Papapanou et al. 1997; Socransky et al. 1994). In comparison to culture techniques, it has several advantages such as high specificity and sensitivity, capacity to detect microorganisms difficult to cultivate and no need of viable bacteria (Papapanou et al. 1997). When compared to polymerase chain reaction (PCR) technique, similar detection degrees have been described (Siqueira et al. 2002). These authors found that major discrepancies between the methods comprised a number of PCR-positive but checkerboard-negative results. Significantly higher prevalence figures for *Treponema denticola* were observed after PCR assessment. The real-time PCR technique is not particularly suitable for the examination of large numbers of samples for large numbers of different species. In contrast, checkerboard DNA–DNA hybridization, permit enumeration of large numbers of species in very large numbers of samples (Sakamoto et al. 2005).

There was no statistical difference in the frequency of subjects positive to the majority of the bacteria studied between JIA and control groups, except for *Fusobacterium nucleatum, Peptostreptococcus micros* and *Campylobacter rectus*, which were detected more frequently in the controls. *Fusobacterium nucleatum* is a fusiforme, anaerobic, gram-negative bacteria, frequently found in the subgingival microbiota of children, adolescents and adults, in both healthy and diseased sites (Darby & Curtis 2001;

Kamma et al. 2000; Moore & Moore 1994; Suda et al. 2003). *Campylobacter rectus* is a motile gram-negative rod whose detection frequency in children's permanent teeth is similar to the present study (40% and 35.7, respectively) (Kamma et al. 2000). Suda et al. (2003) observed that *Campylobacter rectus*, together with *Eikenella corrodens* and *Prevotella intermedia*, were the most frequently detected in healthy children. Similarly, *Peptostreptocccus micros* and *Streptococcus intermedius*, gram-positive anaerobic coccus, are frequently found in the subgingival microbiota of children (Kamma et al. 2000).

We did not observe significant differences between JIA NoAL and AL patients with respect to the subgingival microbiota. However, the frequency of subjects positive to *Porphyromonas gingivalis*, a bacterium associated to more severe and progressive forms of early onset periodontitis (Albandar et al. 1997), was 44.4% in the JIA AL subgroup versus 17.9% in the JIA NoAL subgroup. Similar frequencies of *Porphyromonas gingivalis* (56.4%), as well as of *Actinobacillus actinomycetemcomitans* (22.4%) and *Prevotella intermedia* (74.1%), have been found in patients with incidental periodontitis (Albandar et al. 1997), implicating that these bacteria might also be involved in the occurrence of the observed incipient attachment loss in these patients.

Incipient attachment loss was common in patients with the systemic and enthesitis-related arthritis subtypes of JIA. In the systemic subtype, the inflammatory phenomena are more intense, arthritis and systemic involvement occur at the onset of the disease, i.e., arthritis develops at the same time or is preceded by high spiking fever, rheumatoid rash, hepatosplenomegaly and serositis (Petty et al. 1998). The enthesitis-related subtype of arthritis, inflammation of the bone inserting structures, e.g., tendons, ligaments, joint capsule or fascia, is the childhood equivalent of adult ankylosing spondylitis (Wedderburn & Woo 1999).

The clinical and laboratory outcomes used to describe the severity and activity of JIA included an evaluation of the total number of joints affected, the PGA, the patient's GA, the CHAQ, ESR and CRP (Ravelli et al. 1997). When distinguishing between those with and those without AL, we found that JIA patients with AL tended to have more affected, swollen and painful joints, although only the number of joints with

LOM was statistically significant. This observation and the tendency of higher median values of CRP in JIA AL patients are in agreement with those reported by Mercado et al. (2001) in a group of patients with adult RA. The mean CRP values of our controls were about the same as those of Noack et al. (2001), *i.e.*, 0.90 mg/l.

Mercado et al (2001) suggested that a monocytic hypersecretory state might be a similar tissue destructive mechanism affecting RA/JIA and periodontitis. Challenges to the monocyte/macrophage axis might induce an excessive secretion of proinflammatory cytokines and consequently stimulate the production of degrading enzymes at the articular and periodontal environments. We choose to analyze IL-18 and IL-1β in serum and GCF due to the fact that these cytokines are intimately related to the pathogenesis of RA/JIA and periodontitis (Bresnihan et al. 2005; Maeno et al. 2002; Sugiura et al. 2006). Since an exaggerated neutrophil activity has also been described in these diseases (Figueredo & Gustafsson 1998; Levine et al. 1993; Momohara et al. 1997; Sikora et al. 1994; Sorsa et al. 2004), we opted to analyze this through elastase and MMP-8 in GCF.

We found significantly increased levels of serum IL-18 in patients with JIA, especially those showing AL. IL-18 is a pro-inflammatory regulator of immune responses with similarities to IL-1β and ability to induce TNF-α. These findings in the JIA AL subgroup might infer that these individuals are the more "inflamed" or JIA affected ones and that they could respond more destructively to the bacterial challenge in the periodontal environment. In accordance to this, increased levels of this cytokine have been observed in serum of individuals with JIA and synovial tissue in RA (Bresnihan et al. 2005; Joosten et al. 2003; Maeno et al. 2002). IL-18 has been shown to be related to local and systemic parameters of rheumatological inflammation and to induce the production of IL-1β and TNF-α by monocytes/macrophages (Joosten et al. 2003). We found similar amounts of IL-18 in GCF of JIA individuals compared to controls and in RA subjects compared to controls matched for periodontal conditions, age, gender and smoking habits. Little has been described about the presence and role of IL-18 in the periodontium besides that gingival epithelial cells express it constitutively and produce it under stimulation in vitro (Rouabhia et al. 2002). The ability of other periodontal cells to express and

produce IL-18 need to be addressed in future studies. Recently, Johnson et al. (2005) and Orozco et al. (2006) showed higher concentrations of IL-18 in gingival biopsies and GCF mainly from sites with severe periodontitis. Significant correlations between IL-18 and periodontal measurements, such as PD and AL, observed in this thesis might indicate a possible role of IL-18 in periodontitis.

The levels of IL-1β in GCF from JIA tended to be higher than those in controls, although not statistically significant. The loss of some GCF samples, and consequently the sample size reduction, might have influenced these results. In serum, however, significantly higher amounts of IL1β were found in JIA, compared to controls, which is in agreement with other studies (Altomonte et al. 1992; Yilmaz et al. 2001). There was a tendency for higher proportion of free elastase in GCF samples from patients with JIA, especially those with AL. E+A2MG complex levels were elevated in controls compared to JIA patients. Previous studies have also observed elevated levels of this complex in gingivitis sites of healthy patients (Figueredo et al. 2004). Taken together, these findings indicate a tendency of impaired anti-proteolytic activity in the GCF of this subset of subjects.

At the 2-year examination, the rheumatological status of the JIA patients showed a significant improvement with less clinical signs of inflammation and a lower acute phase reaction. These findings, including a significant decrease in the number of swollen and painful joints, the total number of joints affected and in the ESR levels, might indicate partial or complete remission of the disease. This phenomenon is well described in the literature (Foster et al. 2003; Koivuniemi & Leirisalo-Repo 1999; Minden et al. 2002). Long-term studies have showed that less than half of the patients have active disease at a 16.5-years follow-up, usually at a low level (Minden et al. 2002) and with remission rates that ranged from 45 to 60% (Foster et al. 2003; Koivuniemi & Leirisalo-Repo 1999; Minden et al. 2002).

There were no differences in the periodontal conditions between JIA and control groups at the 2-year examination. In both groups, the periodontitis parameters followed a similar pattern over time. No dental treatment was reported by the individuals of the present study during the follow-up period. Despite of that, we observed a significant decrease in plaque levels in the period. One explanation for this finding could be the Hawthorne effect. This effect is often cited as being responsible for oral health improvements of study participants. It is thought that participating in and fulfilling the requirements of a study alters the subjects' behavior, thereby contributing to the observed improvement (Feil et al. 2002).

The results of Study III study must be interpreted in light of certain limitations. Not all individuals examined at the baseline were available for the year 2 examination. This happened due to an inability to locate these individuals at the telephone numbers and addresses they provided us. This fact probably affected the power of the present study and could have indeed influenced the results.

The amounts of IL-1β on GCF samples from JIA patients decreased significantly after 2 years. There is reason to believe that this decrease was due to the disease remission and/or rheumatological treatment and not due to an improved hygiene since gingival inflammation remained similar. In the second examination, The JIA patients considered active, i.e. given more medication, had lower GCF levels of IL-1β, IL-18 and MMP-8 as compared to the inactive JIA patients. On the whole, these results suggest that the rheumatological treatment clearly positively influenced the periodontal status, which was also confirmed by the findings of no differences between JIA and controls regarding GCF levels of IL-18 and MMP-8.

In GCF, significant correlations were found between IL-1β and both IL-18 and MMP-8 in our study. A correlation between IL-18 and IL-1β was also observed in serum (Study II) and synovial specimens of patients with RA (Joosten et al. 2003). It has been demonstrated that IL-18 induces production of pro-inflammatory cytokines, such as IL-1β (Joosten et al. 2003). Our findings appeared to be in accordance to this principle. MMP-8 is produced by neutrophils and also gingival fibroblasts in response to proinflammatory mediators such as TNF-α and IL-1β (Kiili et al. 2002; Sorsa et al. 2004). Thus, we found a positive correlation between IL-1β and MMP-8 in GCF.

Regarding JIA activity, we did not find differences for IL-18 and IL-1β in GCF in inactive and active subjects, but MMP-8 tended to be higher in inactive patients.

In Study IV, markers of periodontal inflammation, IL-1β, IL-18 and elastase activity, were analyzed in the GCF of individuals with RA and compared them to controls matched for major confounders. In order to be able to assess in the influence of a certain systemic conditions on the periodontal environment, the periodontal status and thus the local degree of inflammation should be comparable. Otherwise, any differences in GCF content could be attributable to the different degrees of periodontal involvement. Additionally, to study only healthy sites would not be feasible since an inflammation is needed to get enough GCF with mediators for analysis. The approach of comparing the GCF inflammatory content of patients with a putative modifier of periodontal inflammation compared to periodontal status-matched controls have been used in other conditions, such as menopause and diabetes (Engebretson et al. 2004; Reinhardt et al. 1994; Salvi et al. 1997). We believe that in the present study the matching of the periodontal conditions, measured by different periodontal parameters, between RA and control individuals was accomplished.

One reason for the similar neutrophilic activity observed in the GCF samples from patients with JIA and controls (Study II) and a possible interpretation for the higher IL-1β levels in the control group and MMP-8 in JIA inactive group (Study III) and lower amounts of IL-1β and elastase activity in the RA individuals (Study IV) could be attributed to the effects of medications in use. The majority of the individuals examined in this thesis were under anti-rheumatic pharmacological treatment (Studies I and II: 76.3%, Study III, 47.1% and Study IV: 88.2%). Most of them were taking NSAIDs, prednisone, MTX and combinations for a prolonged period (Studies I and II: mean disease duration of 4.7 years and Study IV: 12.1 years).

The mechanisms of action of these drugs commonly used to treat RA are only partially understood but it is known that they do modulate the cytokine network and neutrophils in vitro and in vivo (Barrera et al. 1996)**.** NSAIDs produce their therapeutic activities through inhibition of cyclooxygenase (Vane & Botting 1998). Other modes of action include inhibition of IL-1 and IL-6 production by mononuclear cells, as well as having multiple effects on other cellular responses, such as polymorphonuclear cell degranulation, calcium influx and phosphorylation of intracellular proteins (Bondeson 1996). NSAIDs can affect early steps in neutrophil activation, inhibit neutrophil aggregation and superoxide generation (Abramson et al. 1990).

Glucocorticoids affect virtually every cellular and humoral mechanism involved in inflammation, including neutrophils and cytokines. Its mechanism of action is complex and is related to regulation of genes transcription, inhibiting proinflammatory and chemotatic cytokines such as IL-1, TNFα and IL-8 (Boss et al. 1999; Conn 2001; Lee et al. 1988; Loetscher et al. 1994). Corticosteroids have also been shown to downregulate neutrophil function (Perretti & Flower 1993).

MTX has effects on neutrophil superoxide anion formation and adhesion, on the modulation of cytokine responses at a number of levels and on the promotion of apoptosis of activated lymphocytes (Chan & Cronstein 2002; Neurath et al. 1999). The pre-incubation of neutrophils with MTX ablated the mediator release by these cells (Leung et al. 2001). In addition, MTX suppresses macrophage function and modulates IL-1β production (Gerards et al. 2003; Neurath et al. 1999). MTX in combination with prednisone decreases blood levels of IL-1β and IL-6 and inhibits the intensity of free radical-mediated processes in RA subjects (Nowak et al. 1999). Thomas & Carroll (1993) showed that MTX treatment reduced the IL-1β concentration, the number of leukocytes and the number and proportion of neutrophils at the local level, in the synovial fluid of RA patients. These authors also observed that MTX diminished the IL-1β concentration in synovial fluid, whereas IL-1β serum levels were not affected, demonstrating that this drug can alter local cytokine production without affecting systemic production. This could be correlated to the fact that MTX concentration in synovial fluid and bone is roughly 10-fold higher than plasma levels (Bologna et al. 1994).

Collectively, these data show that the drugs used by the RA/JIA patients examined in this thesis have possibly an effect on the inflammatory mediators analyzed. As their effect can be detected not only systemically but also locally in the synovial fluid, we infer that this effect could extend to the GCF environment. Our finding of more IL-1β in GCF from patients with periodontitis but without RA as compared to patients with both diseases (Study IV) corresponds with a similar study by Biyikoglu et al. (2006) who found five times more IL-1β in GCF from non-rheumatic patients with periodontitis.

Incipient attachment loss may develop in adolescents with JIA. The effect of JIA on periodontal conditions is multifactorial and probably includes systemic hyperinflammation, type of disease, disease activity and severity, involvement of temporomandibular joint and hands/fingers joints, medications in use and access to dental care among others. Thus, the prevention and early detection of periodontal tissue destruction in this group is necessary even though this influence on the periodontal environment might be transitory. Poor oral health has a deleterious impact on systemic health. Good oral health is therefore important in order to minimize complications of JIA and its treatment and to reduce the morbidity of caries and gingival disease (Welbury et al. 2003). Dental care and supervision should be routinely provided as part of the JIA multidisciplinary team.

GENERAL CONCLUSION

The studies compiled in this thesis show that adolescents with JIA, especially those more systemically affected, have a worse periodontal condition than controls. However, longitudinally, the effects of disease remission and anti-rheumatic treatment are potentially able to modulate the inflammatory process in the periodontium.

FUTURE DIRECTIONS

This thesis suggests a deleterious effect of JIA on the periodontal conditions during adolescence and that disease remission, disease activity and medications might influence this relationship. The contribution of each of these factors in separate is still unknown. Longitudinal approaches trying to include these individuals in the onset of the rheumatic disease and before treatment is instituted would be of interest.

RA/JIA and periodontitis have a common pathogenesis despite different etiologies. The infiltration of inflammatory cells, cytokines and enzymes orchestrated by immunogenetics, which determine the degree of tissue destruction in the joints and periodontium are probably similar. Recent evidence has shown that in RA and periodontitis the initiation and progression of inflammation may be due to inappropriate response of the anti-inflammatory cytokines (Bozkurt et al. 2006). Further studies are necessary to evaluate such markers, like IL-4, IL-10 in JIA. Havemose-Poulsen et al. (2005) showed that elements of the peripheral blood cytokine profile are shared by individuals RA, JIA and generalized aggressive periodontitis, distinguishing them from subjects free of disease, suggesting that studies on cytokine gene polymorphisms should be conducted. Besides this, additional inflammatory markers such as other proinflammatory cytokines, like IL-17, IL-23 and IL-33, and especially those related to bone destruction, for instance RANKL expression in GCF and gingival tissues, would be a further step in better understanding the relation between RA/JIA and periodontitis.

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