ASSOCIATION BETWEEN PERIODONTITIS AND RHEUMATIC DISEASES

Thesis presented for the degree of Doctor of Philosophy
in the Faculty of Medicine, University of London

Dr Syed Basit Hussain
Periodontology Unit
UCL Eastman Dental Institute

Primary Supervisor: Prof Francesco D'Aiuto
Tertiary Supervisor: Dr Marco Orlandi

DECLARATION

I, Syed Basit Hussain, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature: Date: 15-08-2022

اَللَّهُمَّ خُصَّ انْتَ اوَّلَ ظَالِمٍ بِٱللَّعْنِ مِنِّي

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ثُمَّ ٱلْعَنِ ٱلثَّانِيَ وَٱلثَّالِثَ وَٱلرَّابِعَ

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وَٱلْعَنْ عُبَيْدَ ٱللَّهِ بْنَ زِيَادٍ وَٱبْنَ مَرْجَانَةَ

وَعُمَرَ بْنَ سَعْدٍ وَشِمْراً

وَآلَ ابِي سُفْيَانَ وَآلَ زِيَادٍ وَآلَ مَرْوَانَ

إِلَىٰ يَوْمِ ٱلْقِيَامَةِ

- **ACKNOWLEDGEMENT**

Over the last five years of my PhD, I have grown so much as a person along with a finer understanding of my project and studies. The knowledge and experience I have gained over this programme have no comparison and that I can see clearly now when looking back as I started a PhD at UCL Eastman Dental Institute. My deepest gratitude to my primary supervisor Prof Francesco D'Aiuto and Dr Marco Orlandi who have not only guided me on the journey but also, encouraged and supported me all the way.

Working with Prof D'Aiuto and his passion for research has inspired me also to do my best. His advice and encouragement have been key in achieving this goal despite the obstacles we faced during this journey. I would like to express my deepest gratitude to both my supervisors for their immense support and guidance throughout my PhD.

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I am grateful to my mother Syeda Narjis Khanum and siblings for their immense love and motivation throughout this journey. I would also like to remember and thank my father Syed Sadiq Hussain (Martyr), my brothers Syed Abid Hussain (Martyr) and Syed Athar Hussain (Martyr) for their enormous guidance and enlightenment during the early stages of my life and career. I would also like to mention my late nephew Syed Turab Ali who has been a friend and support throughout this journey. I hope, I have done enough to make them proud.

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Lastly, I would like to thank Dr Jeanie Suvan and my friends Hesham and Shailly for their encouragement and support.

COVID 19 IMPACT STATEMENT

This statement aims to emphasize the significant disruptions encountered during this research programme which mostly impacted on the conduct of the clinical trial and to elucidate the unforeseen challenges posed by the COVID-19 pandemic.

The COVID-19 pandemic, which emerged in late 2019, has had a profound impact on global research endeavours. The unprecedented nature of the pandemic led to widespread disruptions and in particular to the conduct of the clinical research projects linked to this thesis, including but not limited to:

Laboratory and Facility Closures: Research space at UCL including Rheumatology laboratories and facilities were forced to close or operate with limited capacity to comply with public health guidelines and ensure the safety of researchers. These closures hindered access to essential resources and equipment required for the experimental aspects of the clinical studies planned. The closure and stop of clinical research amounted to 18 months in total.

Disruption of Research Activities: The implementation of lockdowns, social distancing measures, and restrictions on laboratory and fieldwork access resulted in substantial disruptions to this research. In addition, the Eastman Dental Institute and Hospital relocated just before the Pandemic closure. This resulted in a period of at least 18 months of no access to clinical facilities and research space to allow the start of the clinical trial.

Shift in Research Priorities: The emergence of COVID-19 prompted a revaluation of research priorities in this study. Attention was redirected towards existing data research and analysis impacting on the research plans, aims of the research and the original potential study.

Ethical Considerations: The pandemic raised ethical considerations in research, particularly regarding the safety of participants and researchers. Protocols and procedures needed adaptation to ensure compliance with health guidelines while maintaining the integrity of the research. The struggle to obtain the ethical approval and to get the space for research became a challenge. There was no availability of space to conduct the trial safely with such high-risk patients. There were many difficulties as the research space and

hospital were located at a distance and patients required multiple visits which were not safe for the SLE patients due to the risk of exposure.

Human Subjects and Data Collection Challenges: Social distancing measures, lockdowns, and travel restrictions impeded the ability to conduct in-person appointments or experiments involving patients with SLE and PD. The limitations in data collection methods compromised the robustness of the study. The study contained SLE subjects who were already immunocompromised and could not risk such exposure during the pandemic. The patients were not willing to participate in the study due to unwanted exposure and risk since their standard hospital visits were only on-line. The highlighted impact of Covid-19 and the shift of research space significantly influenced the action plan for this research.

RESEARCH IMPACT STATEMENT & CONTRIBUTION IN THE FIELD OF MEDICINE & DENTISTRY

The association between systemic autoimmune diseases, particularly SLE (systemic lupus erythematosus (SLE) and Rheumatoid Arthritis (RA), with Periodontitis (PD) is a multifaceted link that transcends the boundaries of dental and rheumatological specialties. Our research impacted on our understanding of these connections, unravelling intricate links that extend beyond mere co-occurrence.

In the realm of SLE, our findings underscore a bidirectional relationship between SLE and PD revealing however that individuals with SLE are not more susceptible to develop periodontal complications than the general population. Similarly, our studies highlight the potential impact of PD on the severity and progression of SLE, emphasizing the systemic repercussions of oral health. By delineating the immunological and inflammatory pathways involved, our work provides critical insights into the shared mechanisms that underlie both conditions. Our investigations into the association between RA and PD yielded some new evidence suggesting a close link between these two inflammatory diseases especially with regards to the impact of PD in exacerbating the inflammatory processes characteristic of RA.

The interplay between PD and rheumatologic diseases has become a focal point in our research endeavours. The aim of this study was to elucidate the implications of our research efforts in understanding the complex relationship between PD and rheumatologic conditions.

Our research has contributed significantly to establishing a robust connection between periodontitis and various rheumatologic diseases, including SLE and RA. Through comprehensive studies and analyses, we have identified common pathways and mechanisms that underscore the correlation between oral health and rheumatologic conditions.

The findings of our research have profound clinical implications, emphasizing the importance of interdisciplinary collaboration between dental and rheumatologic healthcare professionals. The identification of shared inflammatory pathways opens avenues for more targeted and holistic approaches to patient care. Our research will also influence the

development of novel treatment strategies that consider the bidirectional relationship between PD and rheumatologic diseases. Insights gained from our studies contribute to a more nuanced understanding of how interventions in oral health may positively impact the management of rheumatologic conditions, and vice versa.

Understanding the connection between periodontitis and rheumatologic diseases is crucial for public health initiatives. Our research highlights the need for integrated healthcare policies that address both oral and systemic health, promoting preventive measures and early interventions to mitigate the risk of developing or exacerbating rheumatologic conditions.

Our research has contributed to raising awareness among healthcare professionals, policymakers, and the general public about the interconnected nature of oral and systemic health. Education campaigns stemming from our findings aim to empower individuals to prioritize oral hygiene as a preventive measure for rheumatologic diseases.

Despite our strides in understanding the association between PD and rheumatological diseases the challenges persist. Future research directions include exploring additional nuances in the relationship, refining diagnostic tools, and conducting longitudinal studies to better comprehend the temporal aspects of this association.

In conclusion, our research on the interplay between PD and rheumatologic diseases has significantly impacted the scientific community's understanding of this intricate relationship. As we continue to unravel the complexities, we envision a future where our findings translate into improved patient care, enhanced treatment strategies, and informed public health policies that address the intersection of oral and systemic health.

ABSTRACT

Background:

Periodontitis (PD) is a chronic disease defined by the loss of the teeth supporting tissues including bone and periodontal ligament and it is a major cause behind tooth loss. Several studies suggested that PD is more prevalent in patients with Rheumatic Diseases (RD) but inconclusive evidence on a direct association has been reported. Specific plausible biological mechanisms have been proposed as a basis for the association between PD and RD. The aim of this research programme was to explore the nature of the association between PD and RD.

Methods: The research programme methodology included, critical appraisal, observational and experimental evidence generated from the following studies:

(1) Study I: A systematic review aimed at exploring the prevalence of PD in SLE patients (both sex and females only). Differences in periodontal clinical parameters including probing pocket depth (PPD), clinical attachment level (CAL), SLE disease activity index (SLEDAI) scores of SLE in patients with or without PD were also examined.

2) Study II: A systematic review of the association between PD and Rheumatoid Arthritis (RA). The bidirectional nature of their association was assessed with both qualitative and quantitative methodology.

3) Study III: A critical review and observational questionnaire survey linking self-reported PD and Rheumatic Diseases (SLE and RA) with particular emphasis on describing the plausible common mechanistic pathways.

4) Study IV: A secondary analysis of a representative sample of the US population (n= 13,677, NHANES III, 1988-1994) to define the potential association between PD (exposure) and self-reported SLE (outcome) using multivariate linear and logistic regression models. Case-definition, clinical periodontal parameters and periodontal pathogen serum antibodies were used as specific measure of exposure.

5) Study V: A synopsis and protocols for the pilot study with the aim of assessing the impact of Intensive periodontal treatment (IPT) compared to control (CPT) on measures of vascular function in patients with moderate to severe PD and SLE.

Results:

Study I systematic review data demonstrated that SLE diagnosis was associated with greater odds of PD (OR = 1.33, 95% Confidence Interval [CI]: 1.20–1.48). Patients with SLE exhibited no differences in PPD (SMD: −0.09 mm, 95%CI: −0.45–0.27) and CAL (SMD: 0.05 mm, 95%CI: −0.30–0.40) when compared with systemically healthy controls. PD diagnosis was, however, associated with higher SLEDAI scores in patients suffering from SLE (SMD: 0.68, 95% CI: 0.03–1.32).

Study II systematic review data confirmed no substantial effect of RA on PPD and CAL levels of patients with PD when compared to controls, but high degree of study heterogeneity was found. Diagnosis of PD was associated with worse RA disease activity as assessed by an increased DAS28 score of 0.74 (0.25-1.24, 95%CI, $p < 0.001$).

Study III critical review and a questionnaire survey data confirmed that there was no statistically significant difference in the prevalence of self-reported PD in two patients' cohorts (SLE and RA). Self-reported PD had increased CRP levels compared to controls ($p = 0.033$) in patients with SLE whilst in RA patients PD was associated with higher ESR ($p = 0.022$).

Study IV The NHANES data demonstrated that the participants with PD were more likely to have self-reported SLE (OR 2.6 [95%CI 1.1, 6.0]) compared to those without PD. The association was stronger in those with moderate PD diagnosis (OR 6.3 [95%CI 1.4, 28.7]). Participants with higher serum antibody levels of Mm (OR 1.6 [95%CI 1.0, 2.4]) and lower antibody levels of Pg (OR 0.8 [95%CI 0.6, 1.0]) reported higher prevalence of SLE.

Conclusion:

The evidence identified and produced by this study confirmed that PD is closely linked to Rheumatic Diseases such as SLE and RA. Further studies should explore the impact of treatment of PD on the management of Rheumatic Diseases. ~~A~~ pilot RCT as future work will also explore and observe the bi-directional association between the two conditions. Oral health should be promoted in patients suffering from Rheumatic Diseases.

STATEMENT OF CONTRIBUTION

The studies included in this thesis are the outcome of the 4 years of dedication and work at the Periodontology Unit, UCL Eastman dental Institute. All studies that are included in this thesis are original in design and concept. I have performed majority of the work; however, I would appreciate and acknowledge the specific efforts of the staff and collaborators. The detailed contribution of the authors/staff/collaborators are as follows:

➢ Chapter 2

I contributed to study protocol writing, design and concept writing, searches and screening the articles, data extraction, characteristics table, risk of bias assessment, metanalysis, interpretation of the results and manuscript preparation.

Other Contributors:

- Prof Francesco D'Aiuto: Protocol design, interpretation of the results, manuscript preparation.
- Dr Marco Orlandi: Screening, Data extraction, interpretation of the results and suggestions/feedback for manuscript preparation.
- Dr Syeda Ambreen Zehra: Interpretation of the results, evidence table and feedback for manuscript preparation
- Dr Joao Botelho and Dr Vanessa Machado: Statistical analysis

➢ Chapter 3

I contributed to all the parts of the study including research protocols, design and concept writing, searches and screening the articles, data extraction, characteristics table, risk of bias assessment, metanalysis, interpretation of the results and manuscript preparation.

Other Contributors:

- Prof Francesco D'Aiuto: Protocol design, interpretation of the results, manuscript preparation.
- Dr Marco Orlandi: Screening, Data extraction, interpretation of the results and suggestions/feedback for manuscript preparation.

- Dr Syeda Ambreen Zehra: Interpretation of the results, evidence table and feedback for manuscript preparation
- Dr Joao Botelho and Dr Vanessa Machado: Statistical analysis

➢ Chapter 4

I contributed to all the parts of the study including the protocols, design and concept writing, searches and screening the articles, interpretation of the results and manuscript preparation.

Other Contributors:

- Prof Francesco D'Aiuto: Protocol design, interpretation of the results, manuscript preparation.
- Dr Marco Orlandi: suggestions/feedback for manuscript preparation.

➢ Chapter 5

I contributed to all the parts of the study including the protocol, data collection and analysis, manuscript preparation.

Other Contributors:

- Prof Francesco D'Aiuto: Protocol design, interpretation of the results, manuscript preparation.
- Dr Marco Orlandi: Screening, Data extraction, interpretation of the results and suggestions/feedback for manuscript preparation.
- Dr Syeda Ambreen Zehra: Interpretation of the results, evidence table and feedback for manuscript preparation

➢ Chapter 6

I contributed to all the parts of the study including the ethical approval, protocols, design, and concept, expected results, discussion, and conclusion.

Other Contributors:

- Prof Francesco D'Aiuto: Suggestions/feedback for the ethical approval, protocols, design, and concept, expected results and discussion.

- Dr Marco Orlandi: Suggestions/feedback for the ethical approval, protocols, design, concept, expected results and discussion.
- Dr Coziana Ciurtin: Ethics application and Screening of the SLE patients at Rheumatology department UCLH.

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LIST OF ABBREVIATIONS		
•	AAP	American academy of Periodontology
•	ACR	American college of Rheumatology
•	AE	Adverse Event
•	BOP	Bleeding on probing
•	CAL	Clinical Attachment Loss
•	CEJ	Cemento-enamel junction
•	CVD	Cardiovascular diseases
•	CI	Chief Investigator
•	CP	Chronic Periodontitis
•	CRF	Case Report Form
•	CRP	C-reactive protein
•	CRO	Contract Research Organization
•	CPT	Control periodontal treatment
•	DM	Diabetes Mellitus
•	DMC	Data Monitoring Committee
•	ESR	Erythrocyte sedimentation rate
•	FMD	Flow mediated Dilatation
•	GCP	Good Clinical Practice
•	GI	Gingival Index
•	HTR	Heart transplant recipient
•	ICF	Informed Consent Form
•	IDMC	Independent Data Monitoring Committee
•	IPT	Intensive periodontal treatment
•	ISRCTN	International Standard Randomized Controlled Trial Number
•	NHS R&D	National Health Service Research & Development
•	PD	Periodontitis
•	PI	Plaque Index
•	PI	Principal Investigator
•	PIS	Participant Information Sheet

•	**PPD**	**Probing Pocket depth**
•	**PT**	**Periodontal treatment**
•	**RA**	**Rheumatoid Arthritis**
•	**RCT**	**Randomized Controlled Trial**
•	**REC**	**Research Ethics Committee**
•	**SAE**	**Serious Adverse Event**
•	**SLE**	**Systemic Lupus Erythematosus**
•	**SLEDAI**	**Systemic lupus erythematosus Disease activity index**
•	**VPI**	**Visible Plaque Index**

Chapter 1: Introduction

1 Background

The periodontium includes all supporting structures surrounding a tooth and it supports its movement [1]. Periodontal tissues include the gingiva, alveolar bone, cementum, and the periodontal ligament. Gingiva is the only part that is visible clinically in the oral cavity [2].

The most common form of periodontal disorder includes the formation of a non-resolving inflammatory response termed periodontitis. Periodontitis (PD) is an inflammatory disease caused by a dysbiotic dental biofilm, which if left untreated will affect patients' quality of life as it will inevitably lead to the tooth loss [3]. Treatment of PD is based on the removal of the dental biofilm from the root surfaces through manual or powered debridement at various intervals [1].

Previous evidence suggested that PD is not only linked to oral complications, but it is linked to a series of systemic health outcomes [4]. Indeed, evidence suggests that PD and its treatment could impact on the progression and onset of a number of chronic diseases including cardiovascular diseases and diabetes mellitus [5, 6].

Current evidence suggests a possible association between PD and systemic immuno-mediated diseases like arthritis. Amongst the most common systemic inflammatory diseases Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) have been associated with PD and its treatment [7, 8]. The aim of this research programme was to explore the nature of the association between PD and Rheumatic Diseases.

1.1 Periodontitis

PD has been defined as the inflammation of the periodontium leading to clinical manifestations that include clinical attachment loss, periodontal pocketing and alveolar bone loss. Increased pocket depth is one of the most indicative signs of PD. The International Workshop on Periodontal Diseases in 1999 proposed a widely accepted classification for periodontal diseases including PD [9] . However, recently a new classification has been introduced and ~~use~~ used currently for periodontal diseases (figure 1).

1.1.1 Classification of Periodontitis

Individuals suffering from PD may or may not be healthy other than the disease. PD can be localized or generalized according to the sites and teeth involved.

The rate of progression, the pattern of destruction, the age of onset or detection, the relative amounts of dental plaque and calculus and the signs of inflammation are all used to define periodontitis [10].

The British Society of Periodontology (BSP) classified the disease into four stages based on severity (I, II, III or IV) and three grades that are based on disease susceptibility (A, B or C). The stage of PD cannot reduce, because the bone loss is largely irreversible but can lead to further destruction of the bone if left untreated (Table 1 and Figure 1) [11].

Staging of Periodontitis				
	Stage I (early/mild)	**Stage II (moderate)**	**Stage III (severe)**	**Stage IV (very severe)**
1. Interproximal bone loss*	<15% or <2 mm**	Coronal third of root	Mid third of root	Apical third of root
2. Extent	Describe as: • localised (up to 30% of teeth) • generalised (more than 30% of teeth) • molar/incisor pattern			
Grading of Periodontitis				
	Grade A (slow)	**Grade B (moderate)**	**Grade C (rapid)**	
1. % Bone loss / age	<0.5	0.5–1.0	>1.0	
***Maximum bone loss in percentage of root length. **Measurement in mm from cemento-enamel junction (CEJ) if only bitewing radiograph available (bone loss) or no radiographs clinically justified (CAL).**				

Table 1. *Stages and Grading of PD. Modified from [11, 12]*

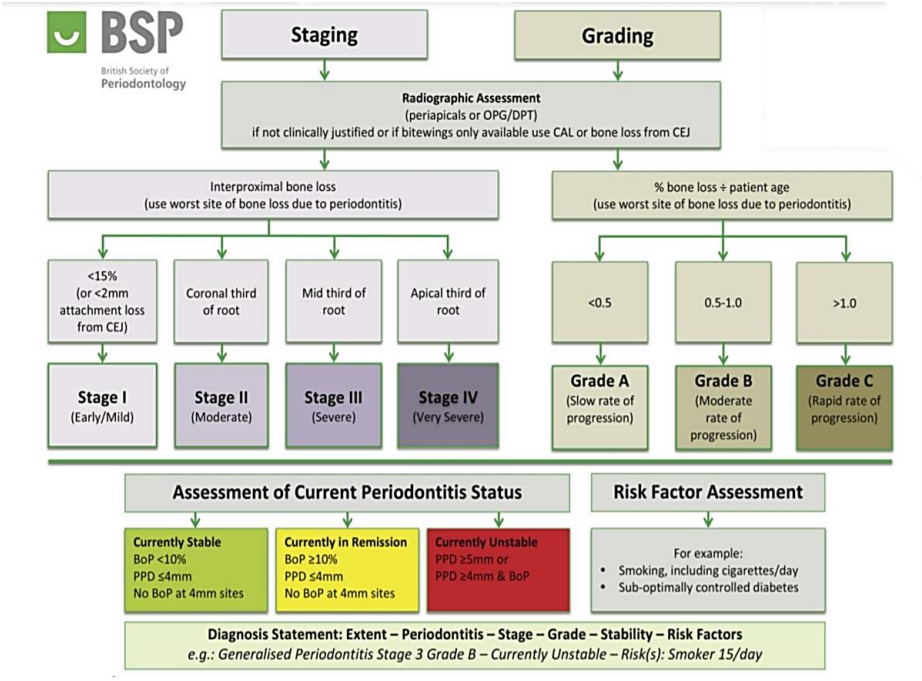

***Figure 1**. Staging and Grading of PD with assessment of risk factor and PD status. Modified from [12].*

1.1.2 Epidemiology

Gingivitis is the most common form of periodontal diseases characterised by a reversible state of gingival inflammation and affecting up to 90% of the population. Periodontitis albeit common is associated to an irreversible and progressive loss of all periodontal tissues. In the 1960s, the most accepted model for the epidemiology of PD was based on the notion that all the patients were equally prone to severe PD. This would suggest that gingivitis eventually progressed into PD along with bone loss and it will eventually lead to tooth loss. However, recent studies shows that PD is not at all age-dependent neither progresses following a linear pattern. Furthermore, its progression depends on a number of risk factors and susceptibility of the host.

Prevalence of PD instead varied over time especially as linked to different case definitions and the latest estimates in Great Britain refer to at least 45% of the population suffering from some forms of PD [13].

1.1.3 Risk Factors

Initiation of periodontal inflammation is influenced by a number of local and systemic factors. Overhanging fillings, occlusal trauma, poor oral hygiene, age, pH of the mouth, food intake and mal alignment are the risk factors that promotes bacterial overgrowth and invasion that may leads to periodontal inflammation. Over the last four decades, potential bacteria always associated with the presence PD include *Aggregantibacter actinomycetemcomitans (Aa)*, *Treponema denticola* (T. denticola) and *Porphyromonas gingivalis (P. gingivalis)*. Evidence from histological analyses of gingival tissues confirmed the presence of these bacteria suggesting them as identification markers for the diagnosis of PD. Furthermore, modifiable/non-modifiable risk factors and predisposing characteristics can also indicate the presence of periodontitis, but they can also alter the progression as well as response to treatment of PD. Based on the notion that PD could have not only a local but also as systemic impact, investigations into the susceptibility to periodontitis have taken on a much wider significance [14].

I. Microorganisms

There are more than 700 different phylotypes found in the oral cavity out of which over 400 is found in the subgingival plaque [15]. The oral flora associated with PD is characterized by several subgroup of micro-organisms, but few pathogens have been associated with severe forms of the disease. *Gram-Negative species* and *Spirochetes* mainly dominate the subgingival plaque in the deeper periodontal pockets [16]. Consistent evidence suggested a major role of *P. Gingivalis* [17] and *Aa* [18] in the onset of adult PD as well as for *Bacteroides Forsythus [19]*, *Prevotella intermedia [20]*, *Peptostreptococcus micros [21]*, and *Fusobacterium nucleatum* [22].

II. Tobacco

Tobacco exposure in the form of cigarettes smoking has been historically linked to worsened periodontal health. Indeed, smoking does impact on the health of gingival tissues, and it contributes to the progression of the PD [23]. Tobacco smoking also magnifies the host response against the pathogens present at the subgingival plaque [24]. Plenty of evidence demonstrated local negative impact of smoking on gingival

tissues, i.e. patients who smoke tend to show apparent less inflammation and gingival bleeding [25]. This is due to the local vasoconstriction caused by the reduced blood flow, clinical signs of inflammation and oedema [26] but greater local destruction [27].

III. Diabetes Mellitus

The oral signs for diabetes (chronic imbalance of glucose metabolism due to progressive reduction of insulin amount or activity) include gingivitis and PD. Several lines of evidence confirm that uncontrolled or undiagnosed diabetes mellitus patients are at the higher risk of PD [28]. This is based on the negative effect of glucose imbalance, formation of large amount of advanced glycation end-products within soft tissues and impact of normal soft tissue homeostasis. However, patients with well-controlled diabetes can maintain periodontal health with lifestyle modification and will respond to the periodontal therapy [29]. Significant evidence over the last 10 years has also confirmed a bi-directional association between PD and diabetes (in particular type 2) with a beneficial effect of the treatment of periodontitis on metabolic control and vascular health in patients with diabetes [29].

IV. Drug Induced disorders

Antimetabolites, various sedatives and tranquilizers, narcotic analgesics, antihypertensives, and antihistamines are known to decrease salivary flow and impact on the oral cavity normal functions. Further various drugs that are available in liquid forms or chewable that may consist of sugar coatings can alter the pH of the oral cavity and promote the composition of the plaque and making it more susceptible to adhere to the tooth surfaces [30]. Furthermore, cyclosporine, anticonvulsants and calcium channel blockers might cause gingival overgrowth and contribute to PD [31].

V. Stress

Stress is known as one of the risk factors of PD, specifically patients presenting with defensive coping behaviours [32, 33]. Stress can be linked with increased secretion of glucocorticoid that supress the immune functioning, increased predisposition to PD or

increased insulin resistance. It has been reported that men with anger issues are 43% more susceptible to have PD rather than those who manifest anger occasionally [34]. Previous studies found that gingival bleeding and tooth loss were associated with financial strains [35] and work stress [34].

VI. Obesity

Obesity is also known as the risk factor associated with PD [36]. This could be strictly linked to the impact of adiposity on the inflammatory response observed in those individuals susceptible to periodontitis. Further original studies focused on the possible dietary intake or excesses/defects to define a possible impact of diet on periodontal tissues health and diseases. Studies have reported that young adults aged (11-18) years have low vegetables and raw fruit intake whereas the adolescents have increased intake of soft drinks and decreased their calcium intake. The link between the dietary intake and PD has been suggested as low Vitamin C and calcium intake have been associated with PD [34].

VII. Osteoporosis

Inconsistent evidence suggested an association between alveolar bone loss and skeletal osteoporosis [37]. It has also been suggested that the postmenopausal osteoporosis can trigger dental osteopenia that can involve the jaws specifically mandible [38]. Osteoporosis has been associated with the prevalence of the PD with sever alveolar bone loss cases in the menopausal female patients [39].

VIII. Haematological disorders

Acute leukaemia results in haemorrhagic gingival growth that can associated with simple soft tissues changes as well as with bone loss [40]. Chronic leukaemia patients may have identical or less severe clinical periodontal symptoms than healthy participants. In addition, chemotherapy which is associated with transplantation of the bone marrow may also have an adverse effect on periodontal health [41].

IX. Host Response

Studies have suggested a complex interaction between the susceptible host and the microbiome in general [42]. The bacterial by-products that include lipopolysaccharides and cytokines cause the stimulation and activation of macrophages within gingival tissues to produce early inflammatory mediators including interleukin (IL)-1 and tumor necrosis factor (TNF). These cytokines activate the fibroblasts that are present within the periodontal tissue resulting in the release of several pro-inflammatory mediators. This results in an imbalance of proteases and their inhibitors such as matrix metalloproteinases (MMPs) and the T-IMPs [43]. The primary component of periodontal matrix is collagen which is highly influenced by the net balance of MMPs. A prolonged and excessive bacterial presence and relative host response will result in large amount of collagen destruction. MMP-(13) further is known to induce bone degradation after the demineralization by the osteoclasts cells [44]. Increased plasma levels of MMPs (8 and 9) has been reported as a distinctive biological feature. It was also observed that periodontal treatment can reduce the levels of MMPs (8 and 9) [45].

X. Pregnancy

Historical evidence reported common changes of periodontal tissues during pregnancy, this been closely linked to the hormonal changes and their impact on soft tissues. On the other hand, an early study by Offenbacher et al. reported more periodontal attachment loss in mothers who presented with pre-term low birth low weight infants as compared to the mother with normal term babies [46]. This and further evidence confirmed the possible impact of PD on the risk of pre-term low birth weight infants and perinatal complications in general. A variety of mechanisms were proposed including the excessive gingival and systemic inflammatory response driven by large amounts of prostaglandins E2 and Tumor necrosis factor (TNF) levels but also by exposure to oral micro-organisms [47].

XI. Age

Several studies reported that the severity and incidence of PD increase with age [48, 49]. According to the seminal studies by Papapanou et al., 70 years old individuals have an annual rate of 0.28 mm of bone loss as compared to just 0.007 mm per year in the 25

years old group [50]. Bone loss and PD's severity associate with age based on the cumulative exposure of periodontal tissues to dental plaque and the oral hygiene history of an individual [51]. Previous studies reported that PD can also present in younger populations and even more severe forms [52].

XII. Gender

Various studies have reported higher prevalence of PD in males than females [53]. The difference in prevalence of the disease among sex is either based on a differential impact of sex hormones onto the dental biofilm and host response as well as a differential distribution of demographic factor including cigarettes exposure [54].

XIII. Socioeconomic Status

The relationship between Socioeconomic status and PD has been proposed by several epidemiological surveys [55]. It has been observed that the patients with lower Socioeconomic status have poor oral hygiene as compared to the patients who have higher education and more secure income.

XIV. Ethnicity and Education

According to the studies by Department of Health Education and Welfare, 1966, reported that the higher the education level, the better the periodontal health. Ethnicity is not defined as a modifiable factor. The nature of the disease might show some variations in the risk factors between populations [56].

XV. Genetics

Several lines of evidence confirm the impact of genetic risk factors in the onset of PD [57]. A study by McDevitt et al. elaborated those adult participants with PD presented with a particular genetic variance (composite IL-1 genotype). The same study also reported that smoking history and IL-1 genotyping were strong risk factors for PD in a private dental practice [58]. Genetic factors have been linked to the onset and progression and the previously termed form of chronic PD [59]. A complex interplay between factors that include behavioural, environmental, education and lifestyle characteristics and host

genetic factors have been considered crucial in the onset of PD. Striking genetic differences between the earlier (Aggressive) forms of PD have been reported but they do not represent a clinical distinctive characteristic [60, 61].

XVI. Systemic inflammation and CRP (C-Reactive Protein)

Various studies have suggested that systemic inflammation as assessed by elevated of CRP is associated with the presence of PD [62, 63]. Whether this is the cause or consequence of periodontitis was initially a matter of debate. Adults with PD were reported by Ebersole et al. to have significantly higher levels of CRP compared to healthy controls [64]. Indeed, it was ever since observed that concentrations of (hs-CRP and IL-6) were significantly higher in patients with PD compared to controls and that treatment of PD decreased their levels. A causal association between periodontitis and systemic inflammation was then proposed.

Elevated levels of CRP indicates that PD could have systemic mediated effects by influencing several chronic systemic disorders that recognize an inflammatory pathogenesis and progression including atherogenic diseases as well as a variety of non-communicable diseases like diabetes, cognitive decline and rheumatic diseases [64].

1.2 Pathogenesis

A number of models and pathways have been proposed to explain the pathogenesis of PD. Soft tissue inflammation linked to both alveolar bone loss and clinical attachment loss are ultimately caused by a sequential interplay between the dental plaque biofilm rich in enzymes, amines and toxins and the soft tissue deranged inflammatory response [65].

1.2.1 Stages of Pathogenesis

Whilst historically gingivitis has been considered the main precursor of PD, this assumption remains unproved due to the lack of clinical evidences [65]. An undisputed role of the dental plaque biofilm in the progression of the disease is a common factor of most paradigms proposed to explain the transition from gingival health to periodontal attachment loss [51, 66] (Figure 2).

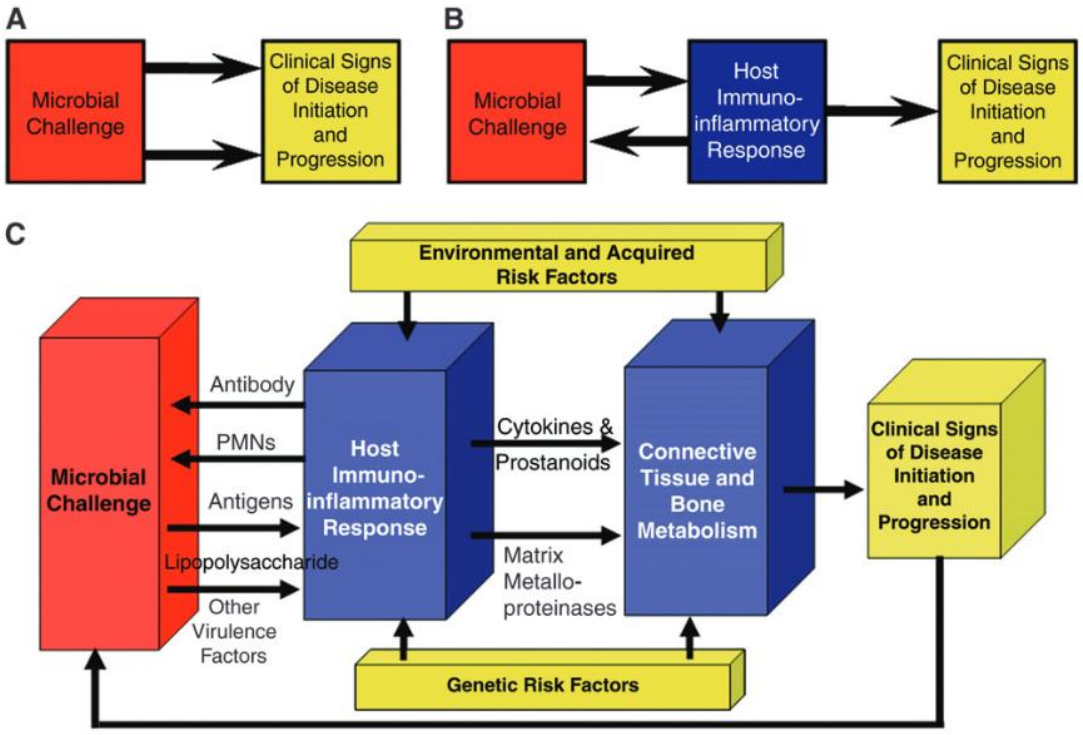

Figure 2. *The adaptation of the conceptual models of PD [67]**.***

a) A previous model representing the role of bacteria in the initiation & progression of the disease. b) Model existed in 1980s emphasizes the auto-inflammatory response contributing to the progression of the disease. c) In 1997, several factors were identified that influence the progression of PD.

Historically several sequential stages in the onset and progression of periodontal inflammation were proposed by Page and Schroeder [68]: including **Initial lesion, Early lesion, Established lesion** and **Advanced lesion**.

1.3 Management of Periodontitis

Diagnosis of PD as early as possible is crucial to avoid the progression of the disease as to prevent the destruction of the healthy periodontium. Management of PD has been recently reviewed and defined depending upon on the stage and grade of the disease. Local or systemic risk factors plays an essential role in progression of the disease and can interfere with the treatment. These factors may include oral hygiene, plaque/calculus

deposits, Bacteria, genetic factors diabetes, occlusal trauma, smoking, stress, patient compliance and iatrogenic dentistry.

Therefore, the practitioner should be able to identify all the risk or influential factors and should be eliminated before initiating the management of the disease [69].

The first step for the management of PD is the early diagnosis of the disease to prevent further destruction of the tissues. Due to the symptomless/painless early stages of the disease, the patient ignores the signs and the disease progresses. However, it is common to ignore the disease at early stages and it can be undiagnosed until it reaches the moderate or advance stage where bone loss and tooth mobility becomes evident. To begin with management of PD, it is essential to diagnosis the condition of the patient and plan the treatment accordingly depending on the various factors that may interfere with the treatment of the disease.

1.3.1 Clinical Pathway for Diagnosis

There are four sequential steps that EFP suggest practitioners should use while examining suspected patients thar are as follows [70]:

- Identify the suspected patient with PD
- Confirmation of the disease
- Identify the stage of the disease
- Identify the grade of the disease

1.3.2 Differential Diagnosis

It is crucial to differentiate PD from other oral conditions that include gingivitis [71], external root resorption lesions, Vertical fracture of the root, cemental tears and cervical decay, other systemic conditions or tumours progressing to the periodontal tissues [72], necrotising PD, local recession (trauma induced), periodontal abscess and endo-periodontal lesions [73, 74].

1.3.3 The EFP S3 Level Periodontal therapy clinical guidelines [75]

Once the patient is diagnosed with PD, it is essential to plan the treatment according to the existing step wise approach that depends on the stage and grade of the disease and each stage include various types of interventions.

After the patient is informed about the diagnosis, the patient should be made aware of the aetiology of the disease, other factors including systemic and local, alternatives for the treatment, additional risks and benefits that includes the option if patient do not want to proceed with the treatment plan. The treatment plan should be discussed with the patients and the outlining of the plan should take place when required and that depends on patient's needs, preferences and clinical findings related to overall health.

The EFP S3 level treatment is divided into three step therapy that are as follows:

1) The first step of the therapy is consisting of a counselling of the patient and to guide them regarding the behaviour change by motivating the patient and encourage the removal of supragingival plaque. Patient should be aware of controlling risk factors and include the following interventional options: Supragingival plaque control, motivation and instructions including [oral hygiene instructions (OHI)] to improve the effectiveness of the therapy and maintenance of the oral hygiene, adjunctive treatment for the inflammation of the gingiva, plaque removal that includes professional mechanical cleaning that includes the professional removal of the supragingival plaque and calculus. It also includes the removal of the plaque retaining factors that can affect the oral hygiene.

   Intervention control also includes all the behavioural changes and elimination of the recognized risk factors that are (cessation of smoking, diabetes control, physical exercise, dietary counselling, and weight loss) for the prevention of the disease and progression. The first step therapy should be introduced to all the PD patient's despite the stage and grade of the disease and the practitioner should evaluate the treatment outcome and review the management of the treatment to enhance the skills in plaque removal and improvise according to the requirement, allowance of the adequate response for making sure the steps of the treatment continue to develop motivation/adherence and evaluate different alternatives to overcome the consequences.

2) The second step in the therapy that is also known as cause related therapy and targets the modulation (reduction/elimination) the subgingival plaque and calculus with subgingival instrumentation. In this second step following interventions are included:

   Usage of secondary chemical and physical agents, Usage of secondary host-regulating agents either local or systemic, Usage of secondary local and systemic antimicrobials. This second step in therapy can be implemented for all the patient's despite of grading and staging of the disease however, it should be applied only in the dentition that has a loss of periodontal support or periodontal pocket formation.

   The outcome of the second step therapy should be evaluated when the periodontal tissues have healed with the therapy. After the evaluation of the second step therapy outcome if no bleeding on probing periodontal pockets (>4 mm) and no periodontal pockets (>6 mm) is not accomplished, the patient should be taken to the third step of the therapy. However, if the goals have been achieved with the second phase of therapy, then the patient should be placed into SPC (supportive periodontal care) programme.

3) The third phase/step of the therapy targets those areas and tissues that have not responded to the second step of the therapy. The third phase should be implemented in the patients if there is a presence of periodontal pockets with bleeding on probing (>4 mm) and deep pockets (>6 mm). The purpose of the third step management is to regain the access to the subgingival instrumentation and targets regeneration or resecting one of the lesions that is crucial for the management of the disease and includes furcation and intra-bony lesions (figure 11).

   The third step of therapy include the following interventions: regenerative periodontal surgery access flap periodontal surgery, repeated subgingival instrumentation (with or without adjunctive therapies) and resective periodontal surgery. These interventions should be subjected to the patient consent and certain analysis of the risk factors and contraindication should be identified and discussed.

   The third step of therapy should be re-evaluated and at the end of the disease therapy, the patient should be placed in the Supportive periodontal care (SPC) programme.

4) Supportive periodontal care (SPC) targets the maintenance of the periodontal tissues by a combined preventive and therapeutic therapies explained in the first and second step of therapies depend on the status of the patient's periodontal and gingival health. In SPC, it is crucial to monitor patients and recall if required. Treatment for reoccurrence of the disease should be reviewed and the diagnosis and treatment plan must be revaluated depending on the patient's needs. Furthermore, following the oral hygiene instructions and healthy lifestyles are the essential parts of the care. In any phase of the therapy extraction of one and multiple teeth can be considered if they are associated with poor prognosis.

1.4 Rheumatic Diseases

Rheumatic disease consists of a diverse group of autoimmune conditions that include RA (rheumatoid arthritis), spondylarthritis i.e., AS (ankylosing spondylitis), PsA (psoriatic arthritis) and the systemic connective tissue diseases include SLE (systemic lupus erythematosus), SSc (systemic sclerosis) and SS (Sjögren syndrome) [76]. All these conditions are similar and exhibit common characteristics such as they are all chronic in nature and cause the destruction and inflammation of the joints and involves the internal organs and that can lead to progressive disability and eventually may cause death. The aetiology of the rheumatological disorders is still unknown and not clear therefore, complete prevention cannot be achieved with the patients. Among these rheumatic diseases, this research programme will primarily focus on two of the conditions that are RA and SLE. RA is known to be the most prevalent disease and it is evaluated that the risk of RA is influenced by the genetic factors that enhances the risk to 50-60% [77, 78]. According to the studies, females are known to be affected more than men with the recorded ratio of (3:1) and for the diagnosis, the mean age is identified between 30-50 years [79]. Various studies have concluded that 19% of the RA patients have become disabled whereas 35% died within 20 years of onset [80, 81]. On the other hand, SLE is known to be the most common type of lupus and is different from the other types due to its multi-system organ effects [82]. SLE can be diagnosed in (20-50) individuals per 100,000 and is more common in females than males specially at the childbearing age. However, studies have suggested that it can affect both males and females at any age

[83, 84]. These two rheumatological conditions have been observed to be associated with PD, suggested by various studies [78, 85]. This study will evaluate the plausible links and bidirectional association between PD and rheumatological diseases specifically SLE and RA.

1.4.1 Systemic Lupus Erythematosus (SLE)

SLE is defined as a chronic autoimmune disease. SLE affects the connective tissues that give flexibility and stability to the body including cartilages and blood vessels. SLE sign and symptoms are very diverse and different in every affected individual. It may affect many systems and organs which includes the joints, skin, central nervous system, hematopoietic system, kidneys, and lungs. SLE can also be defined as a disease that involves the immune system which attacks the body's own organs and tissues.

At the initial stages of SLE the patient will feel tiredness, fatigue, malaise, weight loss, fever and loss of appetite. Majority of the affect patients can also suffer from same joint pain on both sides, weakness, and muscle pain. Along with that skin problems are also very common in SLE. Characteristic feature of SLE associated skin problem is a butterfly rash which is a flat and red rash that appears across the checks and bridge of the nose. It is known as the butterfly rash due to its shape. The rash becomes more pronounced when exposed to the sunlight however, it doesn't hurt or itch. Other problems related to the skin in SLE patients will include calcinosis that is the calcium deposition under the skin, vasculitis (damaged blood vessels) and petechiae. Petechiae are small red spots that are caused by the less cell fragments that are involved in blood clotting known as platelets. Shortage of platelets cell fragments leads to bleeding under the skin hence producing petechiae. Affected individuals may also have alopecia (hair loss), ulcerations of the nose and mouth and less commonly of the genitals.

About one third of the SLE patients develops nephritis (kidney disease). Cardiovascular disease can also occur in patients with SLE including pericarditis (inflammation of the sac like membrane of the heart) and abnormalities of the heart valves that are responsible to control the flow of blood in the heart. Cardiovascular diseases also involve atherosclerosis that is caused by the fat accumulation in the vessels. Atherosclerosis is found in the general population more than the patients of SLE. The characteristic

inflammatory property of the disease can also cause the damage to the nervous system and also involves peripheral neuropathy (weakness of the limbs) and abnormal sensation. Stroke, seizures, cognitive impairment (difficulty in remembering information, learning and processing), depression and anxiety are also common in patients with SLE. SLE patients mostly suffers with exacerbations (condition getting worse) and remissions (relief of condition). The condition of SLE becomes worse over time and destruction of the organs can be life threatening [86].

1.4.2 Epidemiology

Over the last 50 years SLE has found to be more common in industrialized western countries for unknown reasons. In Asia and Africa, the prevalence of SLE is much lower than the western countries. The individuals from Asian or African descent in industrialized western countries are more prone to develop SLE as compared to those of European descent [87] (Figure 3).

In United states SLE affects between 322,000 and 1.5 million. It is difficult to evaluate the exact prevalence because SLE is a multifactorial disease and the signs/symptoms do resemble many other diseases. Diagnosis of SLE can be delayed for years and in some patients, it is possible that the condition has never been diagnosed. Females develop SLE more than nine times than males. Women are more susceptible to SLE during their young age or peaking during the childbearing years. On the other hand 20% of the cases occur around or after the age of 50 [87].

Various studies suggest that factors such as use of tobacco, mixing of ethnic origin and different types of infection that individuals may acquire in different areas can contribute to these differences. For example, malaria which occurs in tropical regions is suspected to be protective against SLE whereas, in the west Epstein Barr virus increases the risk of SLE occurrence. According to the study by Frances et al., it has been reported that the prevalence observed during the study was 4.91 in 100,000 SLE patients per year. However, there was gradual increase of 97.04 in 100, 000 SLE patients in 2012. The peak age was recorded between 50-59 years [88].

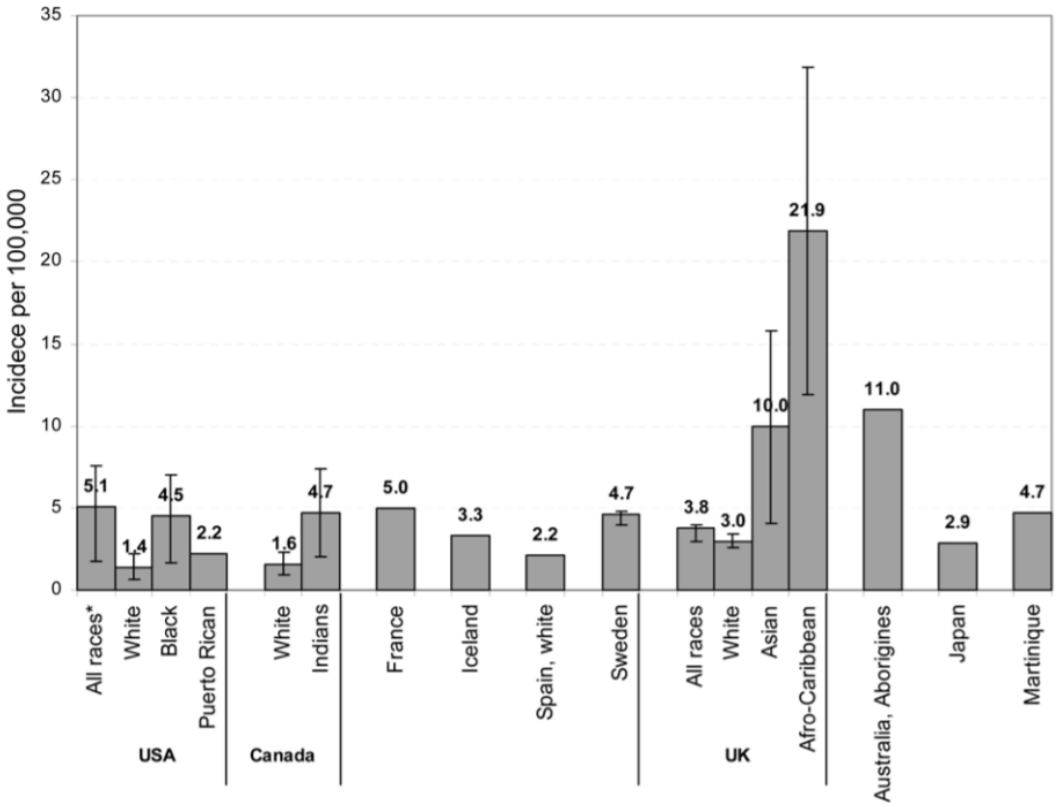

Figure 3: *Incidence of SLE modified from [89]*

1.4.3 Aetiology

SLE is an autoimmune disease characterized by generalised inflammation and destruction of body's own tissues/organs due to the release of immunoglobulin bodies and hyper activation of B cells. This process will inevitably result in multiple organ and tissue damage specifically in the areas where the complexes of immune system deposition occur [90, 91].

A number of genetic variations (polymorphisms) can increase the risk of SLE. Studies suggested that in some cases, even single genes mutations have been found. Most of genes linked to the immune system and its response have been linked to SLE [87].

Further a variety of environmental factors including viral infections, stress, chemical exposures, diet, and sunlight and sex hormones are known to play a vital role in initiating the disorder. Drug exposure is responsible for up to 10% of the SLE cases reported with at least eighty medications ~~been~~ identified as potential triggers of SLE [82].

1.4.4 Pathogenesis

Absence or suppression of T cells is known as one of the pathogenetic mechanisms involved in the onset of SLE [92, 93]. In addition, infections might play a key role in the onset of SLE, particularly in genetically susceptible individuals [94]. An infection can trigger an autoimmune response via formation of self-antigens, molecular mimicry, immune cell activation or through infection-mediated inflammation (figure 4) [95, 96].

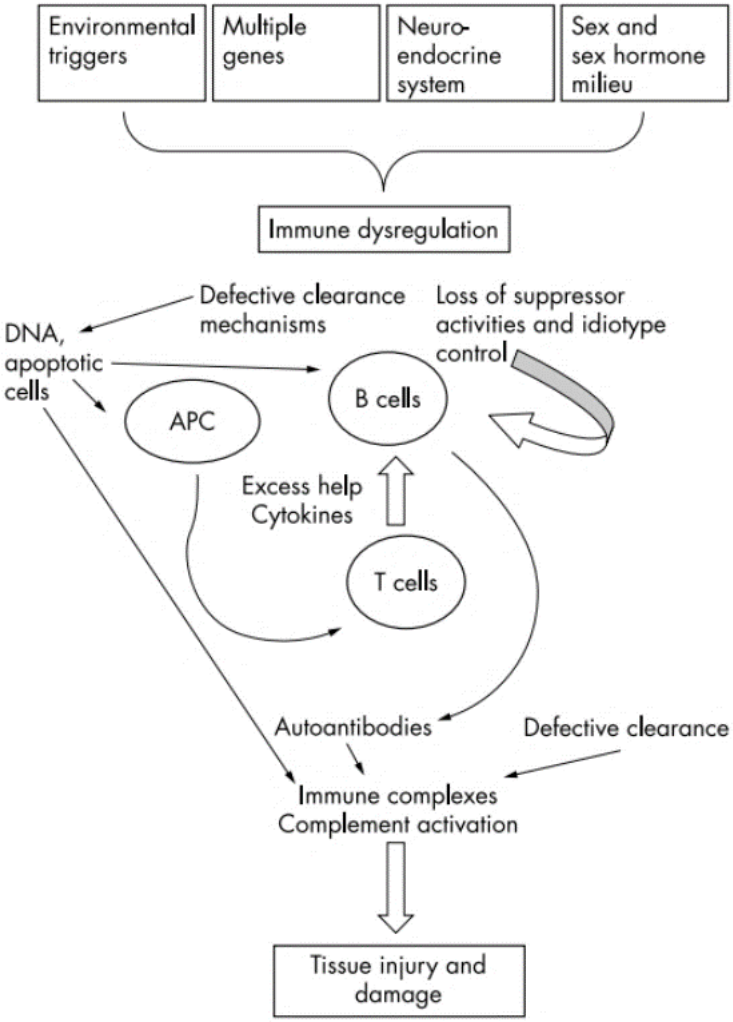

Figure 4. *Pathogenesis of SLE adapted from [97].*

1.4.5 Treatment

SLE is a relapsing and remitting disease and the aim for treatment is threefold:

- Managing acute periods of potentially life-threatening ill health,
- Minimizing the risk of flares during periods of relative stability,
- Controlling the less life-threatening, but often incapacitating day to day symptoms.

Based on the current level of evidence, most of the proposed treatments for SLE are broadly immunosuppressive and carry a significant number of adverse effects. Hydroxychloroquine is the most common drug used for the treatment of SLE, targeting skin disorders, fatigue and joint inflammation or pain. Methotrexate which is classified as an anti-cancer drug is also used for more aggressive form of SLE. Other treatments may involve intra-muscular injections or low dose of steroids for mild diseases and high dose steroids and immunosuppressive therapies are used for the organ involvement and severe forms of SLE [98].

One of the most debilitating complications of SLE includes Lupus nephritis which requires complex renal function management [99]. Current clinical guidelines suggest combining high dose of steroids with cyclophosphamide as the most effective treatment for the renal inflammation and SLE disease [100]. Risk of toxicity and adverse effects limits the use of such treatment approach/combination. Weight gain and osteoporosis are the adverse effects of corticosteroids, whereas cyclophosphamide can cause haemorrhagic cystitis and infertility. More recently, instead of the classic regimen of monthly boluses of 1g cyclophosphamide for 6 months, followed by once every three months for the next 2 years, a "low-dose" (6 fortnightly pulses of 500 mg) regimen has been proposed. The Euro-Lupus trial, published in 2002, showed that the use of this lower dose regimen had better outcomes in terms of infertility risk, with no deleterious impact on renal disease [101]. Azathioprine is another medication often used for the maintenance of the therapy. Mycophenolate mofetil is also used for the treatment of lupus nephritis [102].

Central nervous system involvement also requires immunosuppressive drug treatment that includes azathioprine and cyclophosphamide and can also resolve the serositis and haematological disease. Furthermore, persistent autoimmune thrombocytopenia sometimes requires immunoglobulin infusions.

1.5 Rheumatoid Arthritis [RA]

RA is defined as a chronic autoimmune condition with a higher incidence among females than males. Along with the regional variations, RA has been predominantly observed in the elderly patients with prevalence rate of 0.5 to 1% of the population [103]. RA mainly affects the synovial joints lining and causes the destruction of bone resulting in premature disability, and if left untreated for long periods even death. The clinical parameters of RA include swelling, redness, arthralgia, and limited range of motion. Early diagnosis of the disease is key to avoid the progression of the disease and for expected desirable outcomes that involve reducing the joint destruction, control radiographic destruction and functional disability [104]. However, early diagnosis is dependent on the clinical examination and patient's history followed by blood test and imaging evaluation which makes the diagnosis quite challenging. The early diagnosis can be affected by the regional variations and differences in healthcare systems [105]. In addition, the initiation of disease-modifying antirheumatic drugs (DMARD) is mostly based on the diagnosis, patient's history and examination. However, the treatment of RA depends mostly on the patient's awareness and willingness to undergo therapy from the beginning of the symptoms. With no treatment, the disease will spread aggressively resulting in extra-articular conditions that include pericarditis/pleuritis, small vessel vasculitis, keratitis and rheumatoid nodules (pulmonary granulomas). As there is no definitive treatment for RA, the aim of therapy is to control the disease activity as assessed by clinical and biochemical cluster measures like the Disease Activity Score. The aim of the treatment is to maintain a low disease activity state (LDAS) [106]. There are various scales introduced to measure and evaluate the RA disease activity that are as follows:

- Disease Activity Score using assessment of 28 joints (DAS-28),
- Clinical Disease Assessment Index (CDAI), and
- Simplified Disease Activity Assessment Index (SDAI) [106].

One of the cardinal aspects of RA management is to monitor the disease activity over time and adjusting accordingly the treatment strategy. Corticosteroids and Non-inflammatory steroidal anti-inflammatory drugs (NSAIDs) have proven effective for the treatment of stiffness and pain in RA patients however, they do not control the progression

of RA. DMARDs are known to be an effective therapy, reducing joint deformity and decreasing disease activity [107]. Whilst novel advances are observed in RA, there is no evidence observed for long-term remission of the disease.

1.5.1 Pathogenesis of RA

There are two types of RA based on the presence or absence of the anti-citrullinated protein antibodies (ACPAs). Peptidyl-arginine-deiminase (PAD) is a calcium dependent enzyme that acts as a catalyst for citrullination and resulting into the post-translation modification by transforming a positively charged arginine into a neutral citrulline. 67% of the RA patients have ACPAs which act as a diagnostic reference for early diagnosis and provide an indication regarding the disease progression [108, 109]. It has also been observed that the ACPA +ve patients exhibit more aggressive disease phenotype when compared patients with ACPA -ve patients [110]. Some genetic variants have been reported linked to ACPA (positive and negative) RA patients [111]. In addition, it has been also reported that immune cells respond differently to citrullinated antigens [112]. Methotrexate (MTX) and rituximab [113, 114] have proven to be less effective in patients with ACPA -ve phenotype. Qiang et al. divided the ACPA +ve subset progression into distinctive stages that can occur sequentially or simultaneously in the patients. The stages are as follow [115]:

I. Stage of Triggering

The presence of ACPA is now used as diagnosis criteria for RA because of its high specificity greater than 97%. The occurrence of ACPA (abnormal antibody) is a response against the citrullinated proteins that include α-enolase, fibrin, fibronectin Epstein-Barr Nuclear Antigen 1(EBNA-1), vimentin, histones, and type II collagen. The formation of ACPAs is influenced by environmental and genetic factors. In ACPA +ve patients genes encoding HLA-DR (HLA-DR1 and HLA-DR4) also defined as SEs (shared epitopes) have been associated with the disease [116]. The SEs are known as the primary factor for the production of the ACPA and they also influence the outcome for RA [117]. A lymphoid specific protein tyrosine phosphate, protein tyrosine phosphatase non-receptor type 22 (PTPN22) has been reported in association with ACPA +ve patients [118, 119]. The

PTPN22 is also observed as an inhibitor for T-cell activation and influence the ACPA production. In addition, genetic variation in α1-antitrypsin have been linked with the production of ACPA in patients with RA [120]. It has been reported that ACPA production is associated with hyper responsiveness of type I interferon along with the induction of Th2 immune response and proliferation of B-cell [111, 121].

Family history of RA is one of the most common risk factors. It has been observed that the developing RA was three times higher in relatives of RA patients affecting male and female equally [122, 123]. Environmental factors can influence the ACPA production in RA patients. The combination of genetic and environmental factors triggers the reactivity of the antibodies against the citrullinated antigens in RA patients [124]. Before the joint symptoms occur, it has been observed that ACPAs can be already detected. Noxious agents including silica dust, smoke or nanomaterial derived from carbon can stimulate mucosal toll like receptors (TLRs), which can trigger calcium-mediated PADs and Dendritic (DCs) and B- cells [125, 126]. Further, in relation to the HLA-DRE SE gene, tobacco exposure (cigarettes smoking) could influence certain immune reactions against the citrullinated proteins [127].

Recent accumulating evidence also suggested that the autoimmune reaction triggered in RA could be caused by the common periodontal pathogens including *Pg*, *Aa* and Epstein-Barr virus (EBV). As much as 11% of RA patients were reported to exhibit a history of *Aa* infection. Leukotoxin A secreted by *Aa* can develop pores in the membranes of neutrophils leading to hyper citrullination [128]. *P. gingivalis* can cause the production of ACPA and influence citrullinated autoantigens via several mechanisms. Firstly, the PAD and arginine of the bacteria can cleave proteins at arginine residues and causes the citrullination of the proteins that results in the production of the neoantigens [129]. Another mechanism is linked to the formation of neutrophil extracellular traps (NETs) by *P. gingivalis* [130]. It is also been suggested that *Epstein Bar virus (EBV)* can influence the production of ACPA (B cells) [131].

Evidence of the potential bacterial burden in the pathogenesis of RA comes from studies that suggested that the gut microbiome does contribute to the pathogenetic mechanisms of the disease [132, 133]. Further some studies established a protective role of dietary intake in patients with RA suggesting that (omega 3-fatty acids) have a possible protective role in the onset and progression of the disease [134].

Hormones could contributed to the pathogenesis of RA [135, 136] however, no association with ACPA has been conclusively demonstrated [137].

A myriad of risk factors (Figure 5) could therefore influence the pathogenesis of RA and have been investigated with regards to possible new insight on novel treatments and long term management of the disease [137].

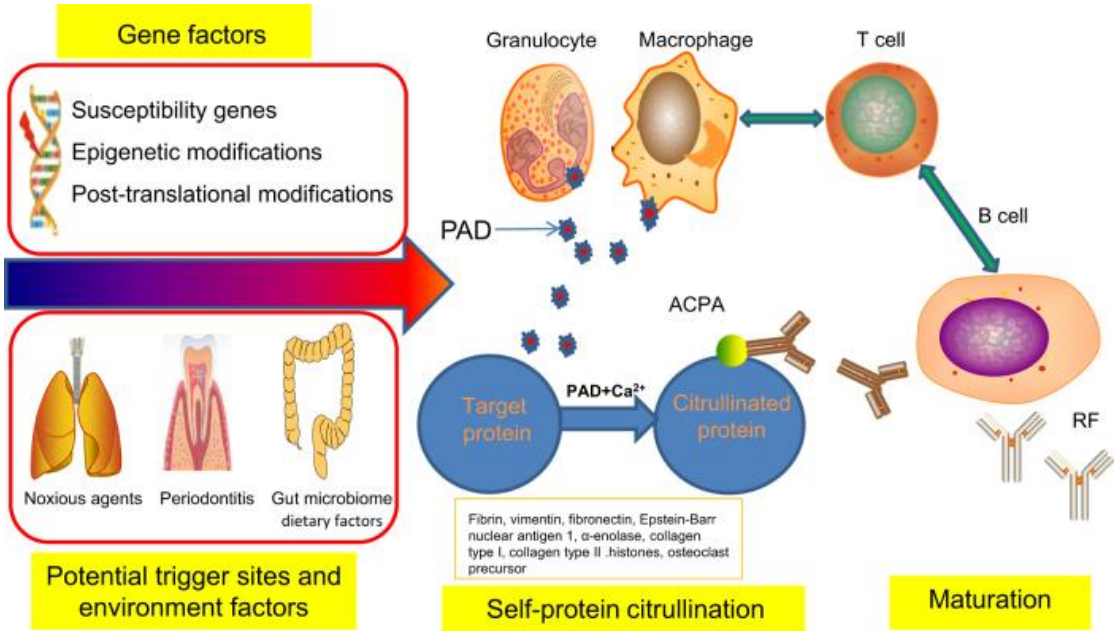

Figure 5. *Pathological mechanism of RA influenced by gene and environmental factors adapted from [115].*

A simplified model of pathogenesis of RA includes genes and environmental factors (Figure 5) closely linked with citrullination of self-proteins and in turn production of autoantibodies. Exposure to various infectious agents including EBV, *P.g and Aa,* noxious agents, dietary factors, and gum microbiome may trigger maturation of ACPA and citrullination of self-protein. As alluded earlier, PAD is known to be a Ca^{2} -dependent enzyme which catalyses the process of citrullination of different proteins. Several

neoantigens might induce activation of T-cells that in turn will promote the ACPA's production through B-cells. This stage is also known as the 'Loss of Tolerance' [137].

II. Stage of Maturation

The most common sites for this stage in RA are bone marrow and lymphoid tissues. The immune response against autoantigens could precede the onset of any clinical symptoms or joints inflammation. During this stage of maturation, an increased production of ACPA and epitope spreading can last for many years before the onset of the RA symptoms [138]. Immunological tolerance can be assessed by the production of ACPA which is responsible of disease symptoms like pain, inflammation, and bone loss in patients with RA [138, 139]. It has also been suggested that the citrullination of the ACPAs is responsible for the differentiation and the activation of osteoclasts and active bone loss Targeted autoantigens plus local microvascular, biomechanical and neurologic factors have been implicated in the progression of the disease [140].

III. Stage of Targeting

The stage of targeting is linked with the site-specific presentation of RA in small joints including the onset of synovitis. Swelling of the joint is the most common consequence of the inflammation of the synovial membrane subsequent to a local immune reaction. Humoral and mediated immunity changes then promote the formation of the ACPAs and can induce the progression to a state of chronic synovitis [140]. Synovial membranes at this stage are infiltrated by macrophages and monocytes which are directly influenced by the ACPA [141]. The production of the pro-inflammatory mediators are usually influenced by the α-Enolase that are present on the surfaces of monocytes and macrophages [142]. An imbalance between M1 & M2 macrophages [142] influences osteoclasts activation and bone resorption in patients with RA [143]. Other inflammatory cells can play an important role in this phase including mast cells contributing to increased expression of TLRs ligands and ACPAs [144], NETosis of neutrophils and activation of the natural killer cells [145]. Several lines of evidence demonstrate a major contribution of the adaptive immune system in the pathogenesis of RA. T-cells and their role in processing and action against antigens have been reported along with the release of pro-inflammatory

cytokines. The abnormal immune response in RA is mediated by (CD4) effector T-cells which can lead to chronic synovitis and influence the local autoantibody production. It has also been suggested that reactive oxygen species could activate pro-inflammatory T-cells in patients with RA [146]. Ultimately a balance between activation of the immune (complex-mediated) complement system and adaptive immune reactions coupled with cytokines networks/self-antigens is key in the further progression of disease (Figure 6) [137].

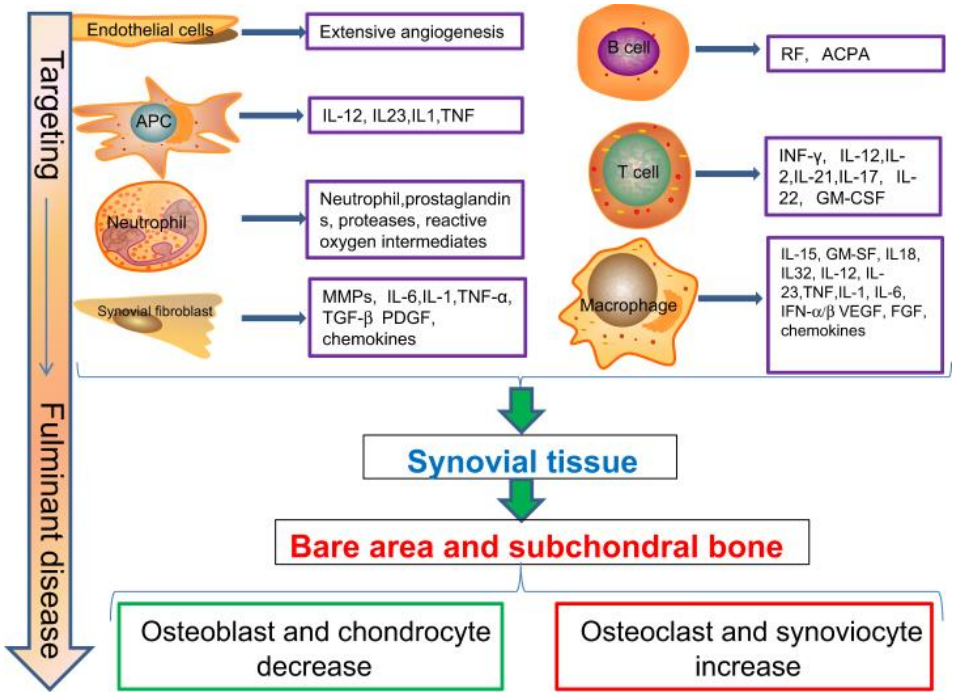

Figure 6. *The transition of the phase of targeting stage to the fulminant disease affecting bone and activates bone resorption adapted from [137].*

Various cells and the cytokines contribute crucially to the progression and development of RA. The synovial membrane can be infiltrated by leukocytes as well as the synovial fluid is enriched of pro-inflammatory mediators. This process is characterized by the fibroblast-like cells reaction to cell-mediated innate immune processes involving monocytes, macrophages, mast cells and dendritic cells. Further, the adaptive immune system also plays an essential role in this paradigm including T-cells and B-cells. All this

biochemical and molecular changes characterize the stage of fulminant RA associated with cartilage damage, erosion of the bone and hyperplastic synovium [137].

IV. Stage of Fulminant

a. Hyperplastic Synovium

Specialized fibroblast like synoviocytes and macrophages forms the Synovium [147] which role is to help maintain a balanced state of the joints as a result of the lubricin and hyaluronic acid release for the function and lubrication of the joints. Fibroblast-like synoviocytes dysfunction is known to be a crucial cause of hyperplastic synovium in RA patients. Alterations to the proliferation of the fibroblast-like synoviocytes due to the failure of contact inhibition that is responsible to produce cytokines and proteinases that include tissue inhibitors of metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs) will contribute to joint destruction. These proteinases create a favourable environment for the T and B cell response which promotes the destruction of the joints [148]. An alternative mechanism proposed for the occurrence of the hyperplastic synovium involves the restriction of cell-apoptosis with alterations of the tumor protein p53 function that leads to the expansion of the synovial lining and destruction of the joint in patients with RA [149].

b. Damage of the Cartilage

One of the main components of the synovial joints is the cartilage tissue consisting of extra cellular matrix (ECM) produced by a specific cell-line, the chondrocytes. ECM contains type II collagen and glycosaminoglycans (GAGs) which confer the tissue its mechanical and protective properties. Cartilage of the synovial joints are severely damaged due to the occurrence of the hyperplastic synovium through the process of invasion and adhesion. Further, ECM saturated by inflammatory signals from fibroblasts-like synoviocytes including MMPs will result in a disorganization of the type II collagen network and biomechanical dysfunction and degradation of the collagenous cartilage matrix [150]. Furthermore, articular cartilage exhibits poor regenerative potential and deprivation of chondrocytes due to apoptosis [151]. This explains the thinning of the joint space that could be observed on radiograph.

c. Bone erosion

RA is characterized by localised (periarticular) and overall systemic bone loss. The production of inflammatory cell infiltrates leading to osteoblasts differentiation and subchondral bone changes ultimately result in bone destruction. It is still a matter of debate whether an auto immune reaction rather than the local inflammatory response is responsible for bone loss and the pathogenesis of RA. Several studies confirmed the role of pro-inflammatory mediators (TNF-α, IL-6, IL-1β & IL-17) in the activation of osteoclasts and decreased bone formation [152]. This mechanism has been targeted with specific medications to contain bone damage and the progression of the disease [153].

The alternative mechanisms involved are based on a local auto-immune reaction. The production of immune complexes and the differentiation of the (Fc-receptor) mediated osteoclasts as well as formation of anti-citrullinated antibodies which target osteoblast activity [129].

1.5.2 Management of RA

Current management of RA involves treatment of the disease symptoms as well as modification of the disease progression. According to the previous evidence, patients with RA who have received delayed DMARDS therapy were more susceptible to develop the space narrowing of the joints and the erosion of the bones [154]. Delayed treatment and poor management of the disease will likely lead to the bony erosions and progression of the disease [155]. Patients with poly arthritis should immediately be referred to a rheumatologist for the confirmation of the diagnosis for RA and an appropriate treatment plan with DMARDS should be introduced to avoid future serious complications (i.e., deformity).

Oral corticosteroids are known to be an effective anti-inflammatory drug that could contribute to the modification of the disease [156]. However, the patients should be aware of the adverse effects linked to chronic use of steroids. Symptomatic management of RA is an essential part throughout the course of the disease that will deal with the primary symptoms of RA that include pain, stiffness and fatigue. Most rheumatologists advise exercise to patients with RA as to help supporting joint function and improve flexibility.

Smoking cessation has also been included in the management of the disease due to its impact on the development of various antibodies [137] (Table 2).

TYPE	DRUG	MECHANISM OF ACTION	ADVERSE EFFECT	REF No.
Conventional synthetic DMARDs	[Methotrexate]	Analog of folic acid	Increased liver enzymes and pulmonary damage.	[157]
	[Leflunomide/ Teriflunomide]	Pyrimidine synthesis inhibitor	Hypertension, diarrhoea and nausea, hepatotoxicity.	[158]
	[Sulfasalazine]	Anti-inflammatory and immunosuppression	Gastrointestinal, central nervous system, and hematologic adverse effect.	[159]
	[Chloroquine /Hydroxychloroquine]	Immunomodulatory effects	Gastrointestinal tract, skin, central nervous system adverse effect and retinal toxicity.	[160]
TNF-α targeted therapy	Infliximab	TNF-α inhibitor	Infection (pneumonia and atypical tuberculosis) injection-site reaction.	[161]
	Adalimumab		Hypertension.	[161]
	Etanercept		Severe /anaphylactoid transfusion reaction.	[161]
	Golimumab			[161]
	Certolizumab pegol			[161]
B-cell targeted therapy	Rituximab	B cell depleting	Infection, hypertension, hypogammaglobulinemia, viral reactivation, vaccination responses.	[161]
	Ofatumumab		Late-onset neutropenia.	[161]
	Belimumab	Inhibitors of B cell function	Severe/anaphylactoid transfusion reaction.	[162]
	Atacicept			[162]
	Tabalumab			[162]
T-cell targeted therapy	Abatacept	CD28/CTLA4 system	Infection, malignancy.	[163]
	Belatacept	CD80/CD86		[163]
Interleukin targeted therapy	Tocilizumab	IL-6 inhibition	Infections (most notably skin and soft tissue), increases in serum cholesterol, transient decreases in neutrophil count and abnormal liver function.	[164]
	Anakinra	IL-1 inhibition	Injection site reactions, infections, neutropenia, malignancy.	[165]
	Canakinumab			[165]
	Rilonacept			[165]
	Secukinumab	IL-17 inhibition	Infections, nasopharyngitis, candidiasis, neutropenia, safety data of mental health is limited.	[166]
	Ixekizumab			
Growth and differentiation factors	Denosumab	RANKL inhibitor	Low Ca2+ and phosphate in the blood, muscle cramps, cellulitis, and numbness.	[167]
	Mavrilimumab	GM-CSF inhibitor	Safety file needs further research.	[168]
JAK pathway	Tofacitinib	JAK1 and JAK3 inhibitor		[169, 170]

	Baricitinib	JAK1 and JAK2 inhibitor	Zoster infection (advice is to vaccinate beforehand) and other potential side-effects should be monitored carefully through further study.	[169, 170]
	Filgotinib	JAK1 inhibitor		[169, 170]

Table 2. *Therapeutic management of RA [115].*

I. Disease-modifying antirheumatic drugs (Biological DMARDS)

DMARDS are defined as the drugs that targets the molecular pathways/specific molecules of the inflammatory processes in RA. DMARDS are known to be effective in RA. Inhibiting agents such as TNF-α along with newer agents targeting B-lymphocytes antibodies including CD-20, IL6, and CD28 have all been proposed in the management of RA [171] (Figure 7).

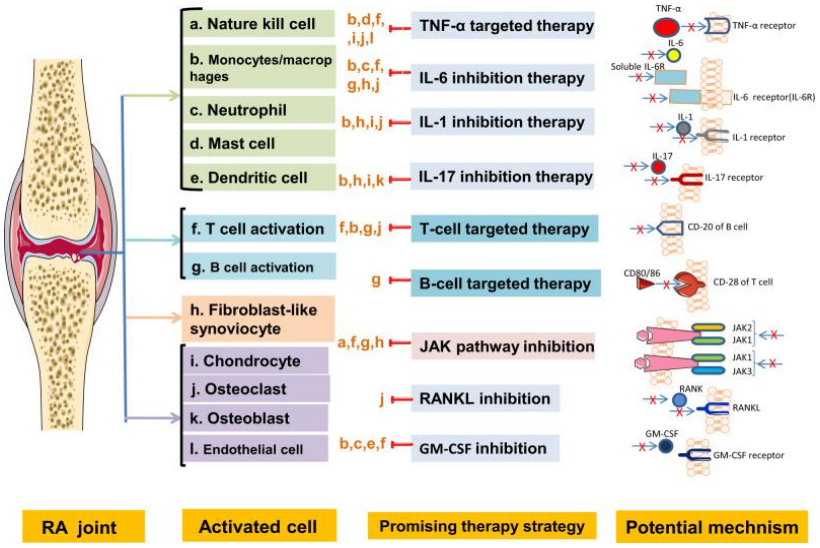

Figure 7. *Pathways for cell/key receptors that are targeted by the therapies. (Nuclear factor-KB ligand, JAK Janus kinase/signal transducers) activated by the RANKL receptor [137].*

1.6 Periodontitis and Rheumatic Diseases

PD and Rheumatic diseases are both inflammatory diseases. Whilst the aetiologies of both disorders can be considered different, as rheumatic diseases are distinctive immuno-mediated disease whilst PD is caused by a dental plaque biofilm, they both result in bone loss and loss of function. Several lines of evidence suggest substantial similarities

between these conditions and possible bi-directional associations. This is further confirmed by the early evidence of positive effect of managing periodontitis on the systemic impact of both SLE and RA. The body of evidence is however still scarce, and interpretation of all studies is further complicated by the lack of definitive studies. This research programme aimed at exploring in greater depth a possible bi-directional association between PD and rheumatic diseases.

1.6.1 Common Etiology

I. Periodontitis and Rheumatoid Arthritis

Recognized risk factors for PD include age, sex, stress, smoking, diabetes, genetic factors, poor oral hygiene, race, ethnicity, and socioeconomic status [7, 172, 173]. According to the National Health and Nutrition Examination Survey (NHANES) data (collected between 1999 to 2004), males (10.65%) are more susceptible to PD as compared to females (6.40%). However, RA is more common in females than males [174]. Smoking and diabetes both modifies the host immune response and are detrimental to destruction of the periodontal tissues [173]. Smoking is known to affect the severity, prevalence, progression, and response to the periodontal treatment [175].

Evidence suggested that there is a significantly higher risk for PD in smokers as compared to non-smokers. The intensity of the risk depends on the duration and rate of smoking [176]. Smokers manifest distinct clinical features with deep pockets, severe clinical attachment loss, higher levels of bone loss and a greater rate of tooth loss [177]. Smoking alters the oral microflora and induces host and immune response that will result in the destruction of the supporting tissues of the tooth (Figure 8) [178].

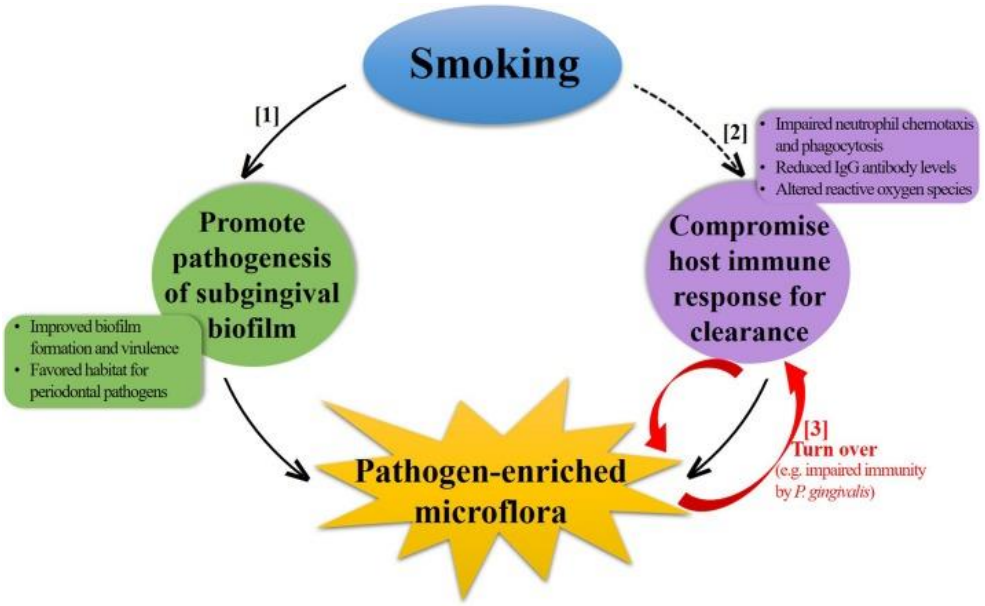

Figure 8. *Representation of the possible mechanisms contributing to the negative impact of smoking on subgingival pathogens [175].*

Smoking is also a common risk factor for RA. A complex interaction between genetic and environmental factors results in a subsequent breakdown of the individual's immune tolerance in patients with RA. Various environmental triggers for RA of which the best defined is cigarettes smoking, in association with genetic factors can increase the disease susceptibility up to 20- to 40-fold [172]. Mechanisms through which cigarette smoking could affect RA include induction of various enzymes such as peptidyl arginine deiminase (PAD) involved in citrullination of peptides at mucosal surfaces (i.e. airways) leading to neoantigens which is recognizable by the adaptive immune system [179].

Diabetes is another risk factor associated with the severity of PD. The underlying mechanisms that might explains the links between diabetes and PD are still unclear however, it does involve the aspects of neutrophil activity, cytokine biology and immune functioning [180]. Evidence suggests the potential two-way relationship between the two conditions which elucidated the high risk of PD in diabetic patients and negative effect on the glycaemic control by the periodontal inflammation [181]. There is a three-fold increase has been observed in the incidences of macroalbuminuria and renal disease in patients with diabetes and PD as compared to the patients with diabetes and without PD.

Moreover, the risk of ischemic heart disease and diabetic nephropathy (cardiorenal mortality) is three times higher in diabetic patients with severe PD as compared to diabetic patients without severe PD [182].

RA and Diabetes has been reported to co-occur in patients [183]. There is a common genetic factor shared by both the conditions is the 620W allele of protein tyrosine phosphatase N22 (*PTPN22*) [184]. In addition, RA is known to be a risk factor for diabetes type 2 according to the observational study which has suggested that patients with high DAS28 score (more than 3.71) were more than 33 times more likely to have diabetes than patients with low DAS28 scores [185]. Hence, for PD Diabetes is one of the risk factors however, RA has been known to be one of the risk factors for Diabetes [24, 27].

Along with the risk factors, it is essential to understand the underlying mechanisms of the disease and the role of bacteria in RA and on the periodontal health. According to the evidence both host response and the pathogen in the plaque contributes to the initiation and progression of PD [186]. The pathogenic bacteria that is commonly found in aggressive PD is *A.a* in adults and young individuals [187]. A. actinomycetemcomitans has been classified into 6 serotypes and it was suggested that few serotypes are associated with periodontal disease rather than the periodontal health. Serotype A and C are more associated with aggressive PD. However, the distribution of the serotypes also depends on the geographic location and ethnicity [188]. Other pathogens associated with PD include *Pg, Treponema denticola*, and *Tannerella forsythia* [189]. *Pg* is a key player in the dysbiotic event resulting in inflammation and periodontal destruction. *Pg* has also been a resource of the virulent factors that contributes to the stimulation of the host responses [190]. In RA, the periopathogenic bacteria *(Pg)* has an influential role by initiating several mechanisms that include citrullination, Netosis, (Th1, Th2, Th17) proinflammatory response and disturbing the osteoclastogenesis by increasing the RANKL, RANK and OPG which will result in bone destruction and systemic inflammation [191].

The genetic factors also have an influential impact on both PD and RA. At least 50% of the individual susceptibility of developing PD is linked to genetic factors [192]. 25 polymorphisms have been related to the intensity of host response to plaque [193]. For the chronic form of RA, (IL-1A, IL-1B, IL-6, IL-10, MMP-3 (chronic form), and MMP-9)

polymorphisms have been significantly associated with a higher risk of developing PD whereas, for the aggressive form (other polymorphisms in the IL-4, IL-8, IL-18, Fc*γ*, COX-2, MMP-2, MMP-3, MMP-8, and MMP-9) polymorphisms had shown no significant association with high risk of developing PD in RA patients [194]. Interleukin (IL) -1 is a proinflammatory cytokine that is related to the severity of periodontal disease. IL-1 α and IL-1 β expression is regulated by the IL-1A and IL-1B genes, respectively [195]. Allele 1 polymorphism in IL-1A was related to PD in African Americans [196]. For IL-1B, the polymorphism was linked to PD in African Americans [197], Caucasians and Chileans [198], but not in North Europeans [199].

It is postulated that the antigens involved in RA, arthritogenic antigens, are modified by a process called citrullination. These citrullinated antigens are recognized by the HLA alleles that have this epitope shared, breaking tolerance, and allowing the formation of antibodies against them. Interestingly, both HLA genes (HLA DRB-1) and some non-HLA genes (PTPN22, 1L23R, TRAF1, CTLA4, IRF5, STAT4, CCR6 and PAD14) [200] have also been linked to autoimmunity to citrullinated proteins (ACPA [anti-citrullinated protein antibody]), as well as smoking. In brief, smoking and possibly other environmental and lifestyle-related factors may trigger ACPA production and the development of ACPA seropositive RA, at least in some cohorts [201].

Epigenetic influences, such as abnormal DNA, dysregulated histones, or expression of microRNAs, can also contribute to disease susceptibility by increasing proinflammatory gene expression. The environmental factors lead to repeated activation of innate immunity, especially at mucosal surfaces, which may take years before the disease becomes clinically evident [202]. The generation of antibodies, such as cyclic citrullinated peptide antibodies (CCP) and rheumatoid factors (RF), which can precede the clinical onset of disease for years, supports the hypothesis of a long-standing aberrant immune responses in RA, eventually leading to clinical disease [203]. What is unique to RA is the propensity of the immune system to recognize neoepitopes created by protein citrullination and the production of specific autoantibodies (ACPAs) [204]. The deposition of these immune complex at the joint location is responsible of inflammatory activation and perpetuation, which eventually could lead to bone and cartilage destruction [205]. The process of citrullination is not specific to this disease as it occurs with almost any

environmental stress factors and also are seen as a common association between RA and PD [204]. Studies have shown common genetic risk factors for RA and PD which include the HLA-DR (Human Leukocyte Antigen), shared polymorphisms, epitope and epigenetic alterations in the cytokine genes [206, 207]. According to another study, indistinguishable changes have also been observed in patients with RA and PD [208].

Common Aetiologies of RA and PD		
PD		**RA**
1	Smoking *Promotes pathogenesis of Periopathogenic bacteria that induces host and immune response.	Smoking *Increases DAS28 *In association with genetic factors can increase the disease susceptibility up to 20- to 40-fold.
2	Diabetes is a risk for PD	RA is a risk factor Diabetes
3	Genetic factor: HLA-DRB1	Genetic factor: HLA-DRB1
4	IL-6 promoter methylation	IL-6 promoter methylation
5	Bacterial pathogens *(P. Gingivalis)* Causes the initiation of the following mechanisms: *Citrullination *Netosis *(Th1, Th2, Th17) proinflammatory response *Increased RANKL, RANK and OPG *Osteoclastogenesis	Bacterial pathogens *(P. Gingivalis)* Amplifies the initiation of the following mechanisms: *Citrullination *Netosis *(Th1, Th2, Th17) proinflammatory response *Increased RANKL, RANK and OPG *Osteoclastogenesis

Table 3. *Summarizing the common aetiologies between PD and RA.*

II. Periodontitis and Systemic Lupus Erythematosus

The exact aetiology of SLE is still unknown. SLE has been linked to a specific risk factor although its clinical presentation poses a serious challenge to clinicians. This is particularly true in patients with serious symptoms such as seizures or psychosis or severe neurological impairment, nephritic or nephrotic syndrome, thrombocytopenia, or haemolytic anaemia [209]. The clinical presentation may be different in different age groups. In children, the disease is more severe at presentation with e.g. acute and severe renal involvement, encephalopathy or haemolytic anaemia, compared to adult-onset of SLE [210]. Common risk factors for SLE may include Smoking, UV exposure, Drugs, stress, Role of Pathogens, and genetic factors. Smoking has been observed as a common risk factor for PD and SLE [211]. Two studies determining the association between SLE, and Smoking have shown that the smokers had significantly higher Disease Activity Index (SLEDAI) scores and a higher odd of thrombotic events [212, 213]. The role of periodontal pathogens *(Aa) as a potential trigger for SLE and RA* have been identified by Konig et al. and Bagavant et al. [201, 214]. In addition, according to Pessoa et al. the pathogens responsible for the initiation and progression of CP might leads to systemic inflammation that includes SLE. Hence, low-grade systemic inflammation that affects SLE disease activity was associated with dysbiotic changes of the pathogens present in periodontal diseases [215].

According to Ramos et al., *HLA-DR2* and *HLA-DR3* (Types II alleles) are one of the most common genetic risk factors related to SLE in Caucasian populations and it has been suggested that these alleles increases the risk for SLE to overall 2 to-3 fold [216].

Also, Kobayashi et al. and Sugita et al. has suggested significant link between FcγR gene polymorphisms and PD risk in SLE [217]. Genetic variants of Fcγ receptor are known to be involved in increasing the susceptibility of both PD and SLE. FcγRIIa is important to initiate phagocytic function on macrophages, monocytes, and neutrophils. It carries histidine (H) and an arginine (R) at amino acid position. FcγRIIa is a poor activator of complement system and suggested to be a predominant IgG sub-class in SLE patients and PD [100]. PD and SLE are inflammatory diseases and share similar inflammatory profiles [218]. Moreover, a potential role of TLR-4 has been observed in a study and has suggested that the specific pathogen-associated molecular patterns produced by

bacteria activates TLR-4 and the stimulation of these molecules results in autoimmune SLE in mice [219].

1.6.2 Common Immune Pathogenesis

I. Periodontitis and Rheumatoid Arthritis

Knowledge of the regulation of immune and inflammatory mechanisms in PD is critical to understand. Bacteria in the plaque mediate the inflammatory response. However, identification has been elusive for the true pathogens. There is specific evidence which suggests that the immune and inflammatory response is exaggerated due to the presence with certain bacteria [220] including *Prevotella nigrescens, Prevotella intermedia,* However, it has been identified that the regulation immune and inflammatory response is based on patient's susceptibility and environmental factors [220]. Innate immunity system that includes the nonhematopoietic cells (epithelial cells) and hematopoietic cells (phagocytes) mediate the initial response to microbial challenge [221]. Neuro peptides also contributes to this reaction and initiate the production of cytokines and chemokines at the site of infection. Moreover, if the infection failed to clear through the response, it will transform from the initial lesion to chronic lesion [221].

In RA, the pathogenesis depends according to the presence or absence of ACPAs (anti-citrullinated protein antibodies). RA is divided into two major subtypes. The presence ACPAs is used as a diagnostic reference for RA for the early and undifferentiated Arthritis and can be detected in approximately 67% of RA patients (figure 9) [109, 222].

As a common immune pathogenesis, RA and PD share several pathobiologic processes that has been suggested by various studies [223-225]. These mechanisms include osteoclast mediated bone resorption, alike cellular participation at the inflammatory sites, matrix metalloproteinase and serum cytokines [172]. Studies have suggested that pathogens of PD have been present in the synovium of the RA patients that will result in the amplification of the inflammatory profile and joint seeding [226].

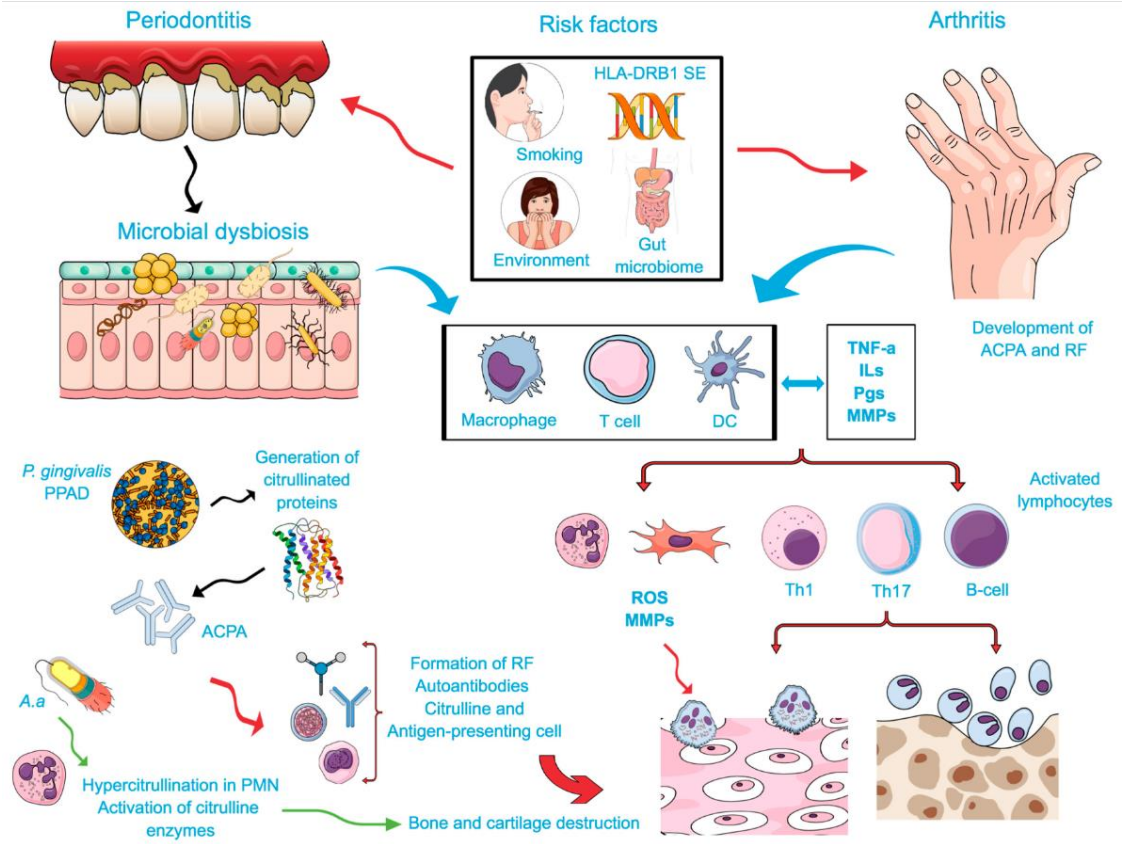

Figure 9. *Linkage and pathogenesis of RA and PD [227].*

II. Periodontitis and Systemic Lupus Erythematosus

SLE onset in considered to be related to two different processes: defects in the clearance of apoptotic cells which leads to the release of autoantigens from necrotic cells as well as inefficient degradation of DNA-containing neutrophil extracellular traps (NETs) leading to accumulation of autoantigens [219]. The immune pathogenesis of SLE is characterized by production of autoantibodies directed against autoantigens (nucleic acids and their binding proteins), which reflects overall a global loss of self-tolerance in genetically predisposed individuals [228].

Both the innate and adaptive immune systems contribute to the pathogenesis of SLE. The aberrant innate immune responses promote tissue injury via release of inflammatory cytokines as well as aberrant activation of autoreactive T and B cells leading to pathogenic autoantibody production and end-organ injury [97]. The immune complexes generated by autoantigens and their cognate autoantibodies could also contribute directly

to the activation of innate immune cells. Autoreactive B cells differentiate into pathogenic memory and plasma cells via the germinal centre, while the follicular helper T (Tfh) cells which reside in germinal centre provide essential signals for B cell maturation and antibody production. This pathogenesis hypothesis has been supported by evidence of expansion of circulating Tfh (cTfh) cell populations in patients with active SLE (Figure 10) [229].

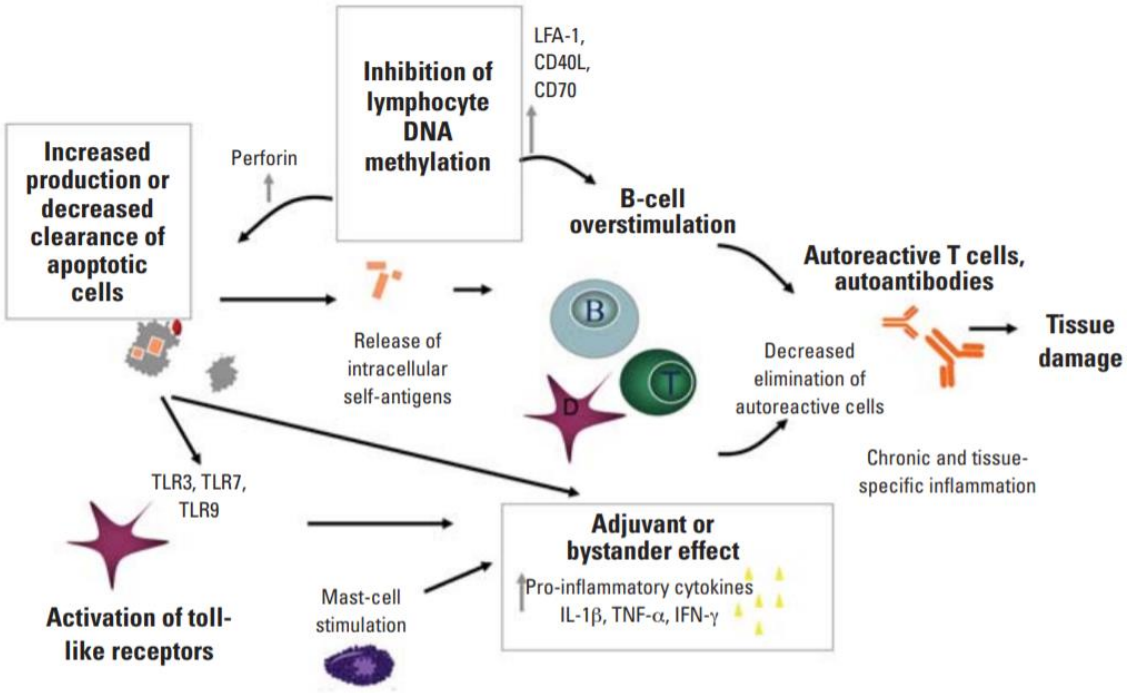

Figure 10. *Auto-immune pathogenesis of* SLE *[230].*
The aberrant innate immune responses promote tissue injury via release of inflammatory cytokines as well as aberrant activation of autoreactive T and B cells leading to pathogenic autoantibody production and end-organ injury.

According to the evidence development of a common immune response has been observed in patients with PD and SLE. High levels of (B cells) have seen in both the diseases with large amounts of immune cells at the affected site. Moreover, there are high production of ANCA (anti-neutrophil cytoplasm antibodies), metalloproteinase modified formation of the of cytokines, increased levels of CRP (C-reactive protein) and TNF-α in the circulation [231]. PD also influences the amount of fibrinogen, cytokines and

CRP (C-reactive protein) and these are associated with the initiation of atherosclerosis. However, the link between PD and SLE is not firmly established. In contrast, the exaggeration of the B cells to the antigenic load existing at the sites affected by PD might demand the stimulus which can activates the B cells resulting in the formation of ANCA (anti-neutrophil cytoplasm antibodies) in SLE patients [232].

Pathogenic bacteria in PD induce over-expression of HSP60 at the affected sites which will result in the stimulation proinflammatory cytokines [233]. The consistent existence of the pathogenic bacteria that induces HSP60 can rise the anti-microbial immunity. However, tenacity of the stimulus, over-expression of the HSP antigens to the invaders will result in a self-immune response stimulated by the host causing the onset of the autoimmune diseases. Therefore, infectious diseases have the ability to trigger autoimmune diseases [234].

There is a significant correlation between PD and SLE pathological mechanisms. The initiation of atherosclerosis, correlation between ANCA and PD in patients with SLE and the over-reaction of the polyclonal B cells signals a strong association between the two conditions. The activation of the polyclonal B cells by the pathogenic bacteria in SLE may influence the number of ANCA (anti-neutrophil cytoplasm antibodies) and might be a significant factor of association between the two diseases [8, 235].

1.6.3 Biological Plausibility between Periodontitis and Rheumatic diseases (SLE and RA)

The biological plausibility between PD and Rheumatological diseases are significantly associated by the inflammatory responses that cause the tissue damage [236]. In PD, the bacteria present in the plaque will be responded by the deployment of the cells and these defence cells will release cytokines such as interleukin-1b, interleukin-6 and tumour necrosis factor-α which will cause tissue destruction. The tissue damage is mainly caused by the production of the collagenolytic enzymes (matrix metalloproteinases) [237].

It has been observed that PD and RA show similarity between pro-inflammatory and anti-inflammatory cytokines. The cytokines (interleukin-1b, interleukin-6 and tumour necrosis factor-α) are known to cause tissue damage, a feature common to both diseases [238].

Evidence suggests a bidirectional association between RA and PD [8, 236]. According to Hochberg et al. a common genetic trait might predispose to RA and PD [237]. Highly polymorphic HLA-DRB1 locus carries the main common genetic factor for RA and PD [239]. PD may be involved in the pathogenesis of RA through bacteraemia, bacterial antigens, presence of inflammatory mediators and immunoglobulins that are present in the serum [8]. Mercado et al. suggested that various microbial factors initiate synovial and soft tissue inflammation which will include CpG motifs, bacterial DNA, lipopolysaccharides and heat shock proteins [238]. In RA, the pathological mechanism behind cartilage and bone destruction are still unknown. However, bone resorption in RA is caused by the activation of cathepsins, MMPs, and osteoclasts which will be accompanied by various cytokines such as Tumor necrosis Factor -α, Inter-leukin-1, and macrophage colony-stimulating factor (MCSF) [173]. The correlation between RA and PD has been explained by three means which include: (1) shared epitope SE-coding HLA-DRB1 allele (genetic susceptibility), (2) microbial dysbiosis at different sites, *P. gingivalis* and the role of ACPA and citrullination, (3) inflammatory response and cytokines [240].

In contrast, characterization of SLE is described as an immune response against the autoantigens affecting women more than men in their second or third decades [238]. It has been suggested by the clinicians that irregular patterns of SLE features has been observed in patients suffering with both RA and SLE disease [241]. It is still debatable that either it is a same immune disease presenting the features of RA and SLE or two distinct diseases with different features [242]. Hypothesis of shared autoimmunity has been supported by the presence of various and identified loci along with increased levels of SLE and RA with the patient genes along with predisposition to immune diseases [243]. Many studies have been conducted to evaluate the association of PD with the rheumatic diseases, especially RA [238] as well as inflammatory bowel disease and glomerulonephritis [8]. Association between PD and SLE has been observed due to the mechanism of tissue destruction. However, the aetiologies for PD and SLE are not similar [8]. Mechanisms that are involved in the deregulation of the innate immune system including the action of pro inflammatory cytokines and phagocyte cells which are IL-1 and IL-18 are majorly responsible for tissue destruction [100]. According to an observational study, prevalence for PD is similar in SLE and RA (approximately 45%) according to the

national estimates (Adult Dental Survey 2009). Hence, it has been concluded that PD could exaggerate an increased inflammatory profile in patients with SLE and RA [244]. According to the current evidence, PD share similar pathological and immunological mechanisms with SLE and RA including microbial response, genetic susceptibility, inflammatory mediators, and alterations in immune reactions. Therefore, this study aimed to observe the association between PD and the rheumatological diseases (SLE and RA).

1.7 Aims & Objectives

Various studies have reported the association between PD and Rheumatological diseases (SLE AND RA). The aim of this study is to look at the association between PD and Rheumatological diseases. The research objectives were set as follows:

1. To quantify the magnitude of association between PD and SLE and the effect of the treatment of PD on SLEDAI score in SLE patients.
2. To investigate the link between PD and RA and the effect of the treatment of PD on the DAS28 score in RA patients.
3. To evaluate the plausible link and pathological mechanisms between PD and rheumatic diseases.
4. To investigate the association between PD and SLE in a large survey of US-based population.
5. To explore the association between SLE and measures of vascular health (brachial artery flow-mediated dilatation) as well as the effect of treatment of PD on the endothelial function in a population of patients suffering from PD and SLE.

1.8 Research Hypothesis

Based on the aim and objectives of the study the main research hypothesis of the study was that "*a causal association between PD and rheumatic diseases exists".*

Specific research hypotheses include:

1 To test the null hypothesis of no association between PD and SLE.

2 To test null hypothesis of no bidirectional association between PD and RA.

3 To test the null hypothesis of no significant plausible links between PD and Rheumatological Diseases (SLE and RA) through a self-reported Oral health questionnaire-based study.

4 To test null hypothesis of no association of PD with SLE in a Large U.S. population-based Survey (NHANES III).

5 To test null hypothesis of no association between SLE and a measure of vascular health (brachial artery flow-mediated dilatation) in patients with SLE and a healthy periodontium compared to patients with PD and SLE.

6 To test the null hypothesis of no effect of Periodontal Treatment (PT) on the endothelial function in a population of patients suffering from PD and SLE.

Chapter 2: Association between Systemic Lupus Erythematosus and Periodontitis: A Systematic review and Meta-analysis

Study I: Association between Systemic Lupus Erythematosus and Periodontitis: A Systematic review and Meta-analysis

2 Introduction

A few studies previously suggested that there was no change in the periodontal clinical parameters of the patients with SLE compared to controls [245]. A systematic review and if possible meta-analysis of all the available evidence on the topic were defined to investigate the potential impact of diagnosis of SLE on the periodontal tissue status.

2.1 Protocols and Registration

The protocol was finalized and subsequently registered on PROSPERO (registration number CRD42018084748). A Prisma checklist (Figure 11) was adopted when reporting rationale and content of this review [246].

2.2 Focused question and Eligibility criteria

A broad research question was chosen to minimize bias when observing the association between PD and SLE: “Is there a bidirectional association between PD and SLE?”, with the following specific questions:

1. “Are patients with SLE more likely to have PD compared with those without SLE?’’
2. “Is the severity of PD influenced by the presence of SLE?”
3. “Does SLEDAI score worsen with the presence of PD?”
4. “Does periodontal treatment improve SLE clinical parameters?”

The following PECO strategy has been employed:

Population: Adults; **Exposure:** Presence of SLE; **Comparison:** Individuals with no SLE; **Outcome(s):** odds ratio of association between SLE with PD, probing pocket depth

(PPD), clinical attachment loss (CAL), effect of PD on SLEDAI score in SLE patients and effect of periodontal treatment on SLE clinical parameters.

2.3 Objectives

2.3.1 Primary and Secondary Objectives

The primary objectives included the prevalence (odds ratio) of PD in SLE patients compared with patients without SLE. Secondary objectives included differences in dental parameters (PPD and CAL) in patients with SLE that was compared with controls. According to the disease exposure and diagnosis, the data derived from the included studies were divided into three groups for PPD and CAL. The mean and standard deviation of PPD and CAL was estimated by using the formula introduced by Hozo et al. [247]. Further secondary objectives included the effect of PD diagnosis on SLEDAI scores in patients with SLE and lastly the impact of PD treatment on SLE clinical parameters.

2.4 Methods

A descriptive protocol was designed for the systematic review and metanalysis which was comprised of the following: aims, objectives, inclusion/exclusion criteria, search and data extraction strategy, risk of bias assessment, and synthesis of extracted evidence.

2.5 Study Design

The aim was to assess the prevalence of PD in SLE patients and compared them to the non-SLE patients. In addition, dental clinical parameters including PPD and CAL were assessed to observe any difference between SLE and non-SLE patients with PD. This study has also investigated the effect of the PD on SLEDAI score including the impact of the PD treatment on SLE clinical parameters.

2.6 Eligibility Criteria for Population

Included study/participants with presence of PD and SLE and/or controls were selected. Studies included participants of age 18 year or above. Definition of SLE was based on the American College of Rheumatology (ACR Criteria) or diagnosis by a rheumatologist.

2.6.1 Inclusion and Exclusion criteria

Included studies were Observational studies (cohort studies, case-control studies, and cross-sectional studies) and experimental studies (randomized controlled trials, controlled clinical trials) including participants older than 18 years of age with SLE diagnosis according to the American college of Rheumatology (ACR) criteria. Review articles, case reports, animal studies, and studies with participants less than 18 years of age were excluded.

2.6.2 Search methodology

Detailed search strategies followed by manual searching were conducted through the following electronic databases: Cochrane Library, MEDLINE, EMBASE, Scopus, LILACS, CINAHL and SIGLE (System for Information on Grey Literature in Europe) until September 2020 with no language restrictions.

Search was done using the following MeSH terms: “(Systemic Lupus Erythematosus OR Lupus Erythematosus OR SLE OR Systemic OR Lupus) AND (periodontal diseases OR gum disease)”. Manual searches through published bibliographies of original manuscripts and reviews within the field of periodontal research, rheumatology over the past 10 years were completed shown in (Table 4 and 5).

MeSH Terms		
MEDLINE	**COCHRANE**	**LILACS**
#1 PD "PD"[MeSH Terms] OR "PD"[All Fields]	#1 MeSH descriptor: [Lupus Erythematosus, Systemic] explode all trees	Subject descriptor lookups (Periodontal diseases or periodontics) and (Systemic Lupus erythematosus) tw:((tw:(PD))) AND (db:("LILACS") AND mj:("Chronic PD" OR "PD" OR "Periodontal Diseases" OR "Periapical PD" OR "Aggressive PD" OR "Gingiva") AND type_of_study:("clinical_trials" OR "case_control" OR "cohort") AND la:("en"))
#2 periodontal disease "periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields]	#2 MeSH descriptor: [Lupus Nephritis] explode all trees	tw:(autoimmune diseases) AND (db:("LILACS") AND mj:("Chronic PD" OR "PD" OR "Periodontal Diseases" OR "Periapical PD" OR "Aggressive PD" OR "Gingiva") AND type_of_study:("clinical_trials" OR "case_control" OR "cohort") AND la:("en"))
#3 periodontal abscess "periodontal abscess"[MeSH Terms] OR ("periodontal"[All Fields] AND "abscess"[All Fields]) OR "periodontal abscess"[All Fields]	#2 MeSH descriptor: [Lupus Nephritis] explode all trees	
#4 aggressive PD "aggressive PD"[MeSH Terms] OR ("aggressive"[All Fields] AND "PD"[All Fields]) OR "aggressive PD"[All Fields]	#3 Any MeSH descriptor in all MeSH products	
#5 chronic PD "chronic PD"[MeSH Terms] OR ("chronic"[All Fields] AND "PD"[All Fields]) OR "chronic PD"[All Fields]	#4 MeSH descriptor: [Aggressive PD] explode all trees	
"periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontology"[All Fields]	#5 MeSH descriptor: [Periodontal Abscess] explode all trees	
#7 periodontal attachment loss "periodontal attachment loss"[MeSH Terms] OR ("periodontal"[All Fields] AND "attachment"[All Fields] AND "loss"[All Fields]) OR "periodontal attachment loss"[All Fields]	#6 Any MeSH descriptor in all MeSH products	
#8 gum disease "gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gum"[All Fields] AND "disease"[All Fields]) OR "gum disease"[All Fields]	#7 MeSH descriptor: [PD] in all MeSH products	
(((((("PD"[MeSH Terms] OR "PD"[All Fields]) OR ("periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields])) OR ("periodontal abscess"[MeSH Terms] OR ("periodontal"[All Fields] AND "abscess"[All Fields]) OR "periodontal abscess"[All Fields])) OR ("chronic PD"[MeSH Terms] OR ("chronic"[All Fields] AND "PD"[All Fields]) OR "chronic PD"[All Fields])) OR ("periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontology"[All Fields])) OR ("periodontal attachment loss"[MeSH Terms] OR ("periodontal"[All Fields] AND "attachment"[All Fields] AND "loss"[All Fields]) OR "periodontal attachment loss"[All Fields])) OR ("gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gum"[All Fields] AND "disease"[All Fields]) OR "gum disease"[All Fields])) OR ("aggressive PD"[MeSH Terms] OR ("aggressive"[All Fields] AND "PD"[All Fields]) OR "aggressive PD"[All Fields])	#8 Add #1#2#3	

#10 systemic lupus erythematosus "lupus erythematosus, systemic"[MeSH Terms] OR ("lupus"[All Fields] AND "erythematosus"[All Fields] AND "systemic"[All Fields]) OR "systemic lupus erythematosus"[All Fields] OR ("systemic"[All Fields] AND "lupus"[All Fields] AND "erythematosis"[All Fields]) OR "systemic lupus erythematosis"[All Fields]	#9 Add #4#5#6#7	
#11 lupus "lupus vulgaris"[MeSH Terms] OR ("lupus"[All Fields] AND "vulgaris"[All Fields]) OR "lupus vulgaris"[All Fields] OR "lupus"[All Fields] OR "lupus erythematosus, systemic"[MeSH Terms] OR ("lupus"[All Fields] AND "erythematosus"[All Fields] AND "systemic"[All Fields]) OR "systemic lupus erythematosus"[All Fields]	#10 Add #8#9	
#12autoimmune disease "autoimmune diseases"[MeSH Terms] OR ("autoimmune"[All Fields] AND "diseases"[All Fields]) OR "autoimmune diseases"[All Fields] OR ("autoimmune"[All Fields] AND "disease"[All Fields]) OR "autoimmune disease"[All Fields]		
#13 lupus nephritis "lupus nephritis"[MeSH Terms] OR ("lupus"[All Fields] AND "nephritis"[All Fields]) OR "lupus nephritis"[All Fields]		
#14 Combine #10#11#12#13 (((systemic lupus erythematosis) OR lupus) OR autoimmune disease) OR lupus nephritis OR ((("lupus erythematosus, systemic"[MeSH Terms] OR ("lupus"[All Fields] AND "erythematosus"[All Fields] AND "systemic"[All Fields]) OR "systemic lupus erythematosus"[All Fields] OR ("systemic"[All Fields] AND "lupus"[All Fields] AND "erythematosis"[All Fields]) OR "systemic lupus erythematosis"[All Fields]) OR ("lupus vulgaris"[MeSH Terms] OR ("lupus"[All Fields] AND "vulgaris"[All Fields]) OR "lupus vulgaris"[All Fields] OR "lupus"[All Fields] OR "lupus erythematosus, systemic"[MeSH Terms] OR ("lupus"[All Fields] AND "erythematosus"[All Fields] AND "systemic"[All Fields]) OR "systemic lupus erythematosus"[All Fields])) OR ("autoimmune diseases"[MeSH Terms] OR ("autoimmune"[All Fields] AND "diseases"[All Fields]) OR "autoimmune diseases"[All Fields] OR ("autoimmune"[All Fields] AND "disease"[All Fields]) OR "autoimmune disease"[All Fields])) OR ("lupus nephritis"[MeSH Terms] OR ("lupus"[All Fields] AND "nephritis"[All Fields]) OR "lupus nephritis"[All Fields])		
Add #9 and #14 (((((((((PD) OR periodontal disease) OR periodontal abscess) OR chronic PD) OR periodontology) OR periodontal attachment loss) OR gum disease) OR aggressive PD)) AND ((((systemic lupus erythematosus) OR lupus) OR autoimmune disease) OR lupus nephritis) 0R (((((((("PD"[MeSH Terms] OR "PD"[All Fields]) OR ("periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields])) OR ("periodontal abscess"[MeSH Terms] OR ("periodontal"[All Fields] AND "abscess"[All Fields]) OR "periodontal abscess"[All Fields])) OR ("chronic PD"[MeSH Terms] OR ("chronic"[All Fields] AND "PD"[All Fields]) OR "chronic PD"[All Fields])) OR ("periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontology"[All Fields])) OR ("periodontal attachment loss"[MeSH Terms] OR ("periodontal"[All Fields] AND "attachment"[All Fields] AND "loss"[All Fields]) OR "periodontal attachment loss"[All Fields])) OR ("gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gum"[All Fields] AND "disease"[All Fields]) OR "gum disease"[All Fields])) OR ("aggressive PD"[MeSH Terms] OR ("aggressive"[All Fields] AND "PD"[All Fields]) OR "aggressive PD"[All Fields])) AND ((((("lupus erythematosus, systemic"[MeSH Terms] OR ("lupus"[All Fields] AND "erythematosus"[All Fields] AND "systemic"[All Fields]) OR "systemic lupus erythematosus"[All Fields] OR ("systemic"[All Fields] AND "lupus"[All Fields] AND "erythematosus"[All Fields])) OR ("lupus vulgaris"[MeSH Terms] OR		

("lupus"[All Fields] AND "vulgaris"[All Fields]) OR "lupus vulgaris"[All Fields] OR "lupus"[All Fields] OR "lupus erythematosus, systemic"[MeSH Terms] OR ("lupus"[All Fields] AND "erythematosus"[All Fields] AND "systemic"[All Fields]) OR "systemic lupus erythematosus"[All Fields])) OR ("autoimmune diseases"[MeSH Terms] OR ("autoimmune"[All Fields] AND "diseases"[All Fields]) OR "autoimmune diseases"[All Fields] OR ("autoimmune"[All Fields] AND "disease"[All Fields]) OR "autoimmune disease"[All Fields])) OR ("lupus nephritis"[MeSH Terms] OR ("lupus"[All Fields] AND "nephritis"[All Fields]) OR "lupus nephritis"[All Fields]))		

Table 4. *MeSH Terms using for the systematic review and metanalyses*

All publication years through April 2018 were included. No languages or year restriction were applied. The bibliographic references were hand searched through the reference list of the identified papers and previous reviews. Online hand searching of issues within the past 10 years of some periodontal, lupus journals was also performed (Table 5).

Periodontal and Medical Journals for Hand-Searching
➢ **Periodontal Journals**
-Journal of Clinical Periodontology
-Journal of Periodontal Research
-Journal of Periodontology
-Journal of Dental Research
-Periodontology 2000
➢ **Rheumatology Journals**
-Annals of Rheumatic diseases
-Arthritis and Rheumatology
-Rheumatology
-Journal of Rheumatology
-Nature review of Rheumatology

Table 5. *Periodontal and Medical Journals for Hand-Searching*

2.7 Study Selection

Titles and abstracts were screened and independently assessed for eligibility with another reviewers (YL). Full-text papers meeting the inclusion criteria were evaluated in duplicate by the same reviewers. Any disagreement regarding their eligibility was resolved by discussion with a third person (MO). The agreement between the reviewers was assessed by Kappa statistic.

2.7.1 Data Extraction and Synthesis

Independent data extraction was completed by two reviewers (BH and YL) and discrepancies were resolved through discussion with a third reviewer (MO) if necessary. The following information was retrieved from all the eligible studies: 1) Year of publication and Country of publication; 2) Study design; 3) Sample size; 4) Periodontal criteria; 5) Periodontal parameters: CAL, PPD, bleeding on probing (BOP), visible plaque index (VPI) and gingival index (GI); 6) SLE diagnosis based on the ACR criteria; 7) Main findings; 8) Published conclusion (Table 6). When data was not located in the manuscripts, every effort was made to contact the authors and requesting the missing information.

Evidence Table for Case-Control Studies

1st Author, Year, Country, Title	Study Design	Sample Size	Periodontal Criteria	Periodontal Parameters	SLE Diagnosis	Main Findings	Published Conclusion
Mutlu et al. 1993 [248], United Kingdom **Gingival and periodontal health in systemic lupus erythematosus**	Case-control	Cases group (n=27): SLE patients Control group (n=25): healthy subjects	Plaque indices (PI) and Gingival Bleeding indices (GI) was assessed by the method of SILNESS & LOE and PPD with a calibrated periodontal probe.	GI, PPD, and PI	ACR criteria (1982)	It has been observed that SLE had significantly (p>< 0.001) lower PPD as compared to healthy controls. Calculating the mean of PPD in SLE patients who were on immunomodulatory drugs showed that the PPD in SLE patients was not statistically affected by immuno-modulatory drugs (immunomodulators: n =22, mean PD= 1.23; no immunomodulators; n = 5, mean PD= 1.5).	Patients with SLE presented similar GI and PI but significantly lower PPD compared to controls.
			Three characteristics were set for the diagnosis of PD: 1) Presence of at least 3 PPD than 4 mm, on 2 separate sides of the mouth. 2) Bleeding on gentle probing in affected sites; and 3) Evidence of bone loss manifested as the distance between the crest and >2 mm, or presence of bone loss seen at the crest of the interproximal alveolar process with or without involvement of the lamina dura	PPD, CAL, BOP.	ACR criteria (1982)	18 of 30 SLE patients (60%) had PD and 12 had a healthy periodontal condition. 19 individuals (63%) in this group were ANCA positive. 15 of 18 (83%) patients with PD were ANCA positive and 3 (16.6%) were negative. 4 of 12 patients (33.3%) without PD were ANCA positive and 8 of 12 (66.6%) showed no reactivity for these autoantibodies. Significant association (P < 0.005) between PD and ANCA was observed.	High occurrence of PD in SLE patients.
Meyer et al. 2000 [249], Germany **Oral findings in three different groups of immunocompromised patients**			The decayed, missing, filled tooth index (DMFT) and the missing tooth index (MT) were determined using the World Health Organization criteria. VPI and GI were used to assess the patient's periodontal status. The vertical periodontal bone loss was also recorded. Vertical bone loss was determined according to	GI, VPI, DMFT	ACR criteria (1982)	Oral mucosal lesions were found in 49.6 % of the immune-comprised patients however 26% were found in controls. No statistically significant findings were observed between the underlying causes of immune-suppression and oral lesions. be significant in the assessment of oral health. Leukaemia patients showed high scores of periodontal indices (p=0.05) which wasn't age related.	No significant association found between oral conditions and immunological diseases.

			a modified method of Albandar et al.			Average bone loss in SLE patients was higher than in HTR and control patients, despite the overall old-age in the HTR.	
Kobayashi et al. [100], 2003, Japan **Risk of Periodontitis in Systemic Lupus Erythematosus Is Associated with Fcγ Receptor Polymorphisms**	Case-control	Cases group 1 (n=42): SLE + PD patients Cases group 2 (n=18): SLE Control group 1 (n=42): non-SLE subjects with PD Control group 2 (n=42): healthy	1) Number of missing teeth 2) PPD expressed as the mean distance from the free gingival margin to the bottom of the pocket was used to divide patients into 3 groups (<4 mm, 4 to 6 mm, and >6 mm 3) CAL expressed as the mean distance from the CEJ to the bottom of the pocket, was used to divide patients into 3 groups as with PPD 4) Supragingival plaque accumulation 5) BOP 6) Bone loss	PPD, CAL, BOP, FMPS, and % alveolar bone loss (X-rays)	ACR criteria (1997)	The SLE and PD group had more mild levels of periodontal destruction than the H/PD group (p< 0.01). There was a significant difference in the distribution of FcγRIIa genotypes between SLE/P and H/H groups (P = 0.004). A significant overrepresentation of the FcγRIIa-R131 allele was found in the SLE/PD group compared to the H/H group (SLE/PD versus H/H: odds ratio [OR] 3.13, 95% confidence interval [CI] 1.46-6.77, P = 0.0013). The prevalence of PD was found to be 70% in SLE patients. The FcγRIIa-R131 allele was also found to be overrepresented in the SLE/PD group	These results show the FcγRIIa-R131 allele to be associated with PD risk in SLE patients.
Kobayashi et al. 2007 [90], Japan **The Combined Genotypes of Stimulatory and Inhibitory Fcγ Receptors Associated With Systemic Lupus Erythematosus and Periodontitis in Japanese Adults**	Case-control	Cases group 1 (n=46): SLE + PD patients Cases group 2 (n=25): SLE patients without PD Control group 1 (n=58): non-SLE subjects with PD Control group 2 (n=44): healthy	1) Number of missing teeth 2) PPD expressed as the mean distance from the free gingival margin to the bottom of the pocket was used to divide patients into 3 groups (<4 mm, 4 to 6 mm, and >6 mm) 3) CAL expressed as the mean distance from the CEJ to the bottom of the pocket, was used to divide patients into 3 groups as with PPD 4) Supragingival plaque accumulation; and 5) BOP 6) Bone loss	PPD, CAL, BOP, FMPS, and % alveolar bone loss (X-ray)	ACR criteria (1997)	A significant overrepresentation of the R131 allele of stimulatory FcγRIIA and the 232T allele of inhibitory FcγRIIB was observed in SLE+PD group compared to the H group (P = 0.01 and P = 0.0009, respectively). The combination of FcγRIIA-R131 and FcγRIIB-232T alleles had a strong association with SLE and PD (SLE+P group versus P group: P = 0.01, odds ratio: 3.3; SLE+P group versus H group: P = 0.0009, odds ratio: 11.2). SLE patients with combined FcgR risk alleles showed more severe periodontal tissue destruction as compared to other SLE patients. The frequencies of IL-1 polymorphic alleles were too low to assess the association with SLE or PD.	The combination of stimulatory FcγRIIA and inhibitory FcγRIIB genotypes may increase susceptibility to SLE and PD in the Japanese population.

Wang et al. 2015 [250], Taiwan **B2-Glycoprotein I-Dependent Anti-Cardiolipin Antibodies Associated with Periodontitis in Patients with Systemic Lupus Erythematosus**	Case-control	Cases group (n=53): Control group (n=56) healthy subjects	Participants who presented with ≥20% of tooth sites with PPD ≥4 mm or ≥CAL 4 mm were defined as patients with PD.	PPD, CAL	ACR criteria (2000)	The prevalence of PD and percentage of sites with PD ≥4 mm and CAL ≥4 mm was higher in both the active and inactive SLE patients than those in healthy controls. hs-CRP, ESR, and anti-dsDNA were significantly higher in the patients with active SLE than in the patients with inactive SLE. Serum Anti-CL and Anti-b2GPI Antibody Levels. Both patients with active SLE and those with inactive SLE showed significantly higher serum anti-CL and anti-b2GPI antibody titers when compared with healthy controls (P<0.05).	Elevated anti-CL and anti-b2GPI antibody levels were associated with PD in patients with SLE
Marques et al. 2016 [250], Brazil **Salivary levels of inflammatory cytokines and their association to periodontal disease in systemic lupus erythematosus patients.** **A case-control study**	Case-control	Cases group 1 (n=30): SLE + PD patients Cases group 2 (n=30): SLE patients without PD Control group 1 (n=27): non-SLE subjects with PD Control group 2 (n=27): healthy	Patients who presented ≥CAL P 6 mm in 2 teeth and 1 or more sites with PPD ≥5 mm was considered for diagnosis of chronic PD.	PPD, CAL, GI, and PI	ACR criteria (1982)	There was no statistically significant difference regarding the age of the patients between the study groups ($p > 0.05$). Concerning the periodontal parameters, PS, NIC, ISG and IPV there was a similarity between the LS and S groups ($p > 0.05$) which showed less periodontal damage than the CP (LP and P groups) ($p < 0.05$). Among the groups with PD, P group showed higher periodontal clinical parameters than the LP group ($p < 0.05$).	Patients with SLE and PD had lower periodontal parameters values compared with healthy subjects with PD
Calderaro et al. [251], 2017, Brazil	Case-control	Cases group (n=75): SLE patients	The definition of PD was based on the criteria	PPD, CAL, and BOP	ACR criteria (1982/1997)	No differences between groups in terms of PPD and CAL.	CP seemed to develop earlier in SLE patients and was associated with
Is chronic periodontitis premature in systemic lupus erythematosus patients?		Control group (n=75): healthy subjects	proposed by Eke et al. (2012)		SLE activity and damage assessed by SLEDAI and SDI,	The prevalence of PD was similar in both groups. No association between diseases was found (OR=2.92; 95% CI:0.99-5.98, P=0.06)	features related to organ damage.
Corrêa et al. 2017 [252], Brazil **Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status**	Case-control	Cases group 1 (n=35): SLE + PD patients Cases group 2 (n=17): SLE patients without PD Control group 1 (n=28): non-SLE subjects with PD Control group 2 (n=24): healthy	PD was defined as the presence of two or more interproximal sites with probing depth ≥4 mm or one site with probing depth ≥5 mm.	PPD, CAL, BOP, and PI	ACR (SLEDAI 2000) SLE activity and damage assessed by SLICC damage index for SLE (SDI) respectively	The prevalence of CP in healthy Brazilians is consistent with previous reports [28–30]. The presence of PD at a younger age in SLE patients (40.5 ± 10.1 years) compared to controls (46.3 ± 13.2, $p < 0.05$).	SLE patients exhibit a higher prevalence of periodontal disease and increased periodontal disease severity.
Wu et al. 2017 [253],	Case-control	Cases group 1 PD patients with SLE	In this study, patients who had one or more	N/A	ACR classification	A statistically significant association between a history of PD and newly diagnosed SLE was	

Taiwan **Association between a history of periodontitis and the risk of systemic lupus erythematosus in Taiwan: A nationwide, population-based, case-control study**		n = 7204 Control group n = 72,040 non-SLE patients	out-patient visit before the index date which diagnosed them as having PD and who were concurrently treated with antibiotics, or dental scaling ≥3 times per year by certified dentists, were identified as patients with a history of PD.		criteria for SLE (1997)	observed (OR, 1.21; 95% CI, 1.14–1.28; p-value, <0.001). The link between dose and time-dependent and was found to be strongest when the interval between the last PD-related visit and the index date was less than three months (OR, 1.83; 95% CI, 1.61–2.09; p-value, <0.001).	The association between PD exposure and SLE risk was consistently significant among subgroups stratified based on age, gender, or DM status. PD severity positively correlated with more PD-related visits. In addition, DM to be an important risk factor for PD.
Corrêa et al. 2018 [254], Brazil **Impact of systemic lupus erythematosus on oral health-related quality of life**	Case-control	Cases group 1 (n=75) SLE Patients (n=78) non-SLE Patients (control group)	PD was defined as two or more inter-proximal sites with CAL of 3 mm or greater, and two or more inter-proximal sites with PPD of 4 mm or greater (not on same tooth) or one site with PPD of 5 mm or greater (Eke et al. 2012)	PPD, CAL	ACR (SLEDAI 2000) SLE activity and damage assessed by SLICC damage index for SLE (SDI) respectively	No significant differences were found in other habits and clinical parameters evaluated such as smoking, flossing, salivary flux, PD, decayed and filled teeth. Patients with SLE showed worse oral health-related quality of life than controls (P = 0.011). The significant difference was on individuals' physical disability (P = 0.002). Prosthesis has been suggested as a determinant to negatively affect the oral hygiene (P < 0.05).	No correlations found in SLE patients with PD. SLE patients had more missing teeth as compared to patients with no SLE.
Pessoa et al. 2019 [215], Brazil **Host-Microbial Interaction in Systemic Lupus Erythematosus and Periodontitis**	Case-control	Total Female subjects (n=91) SLE-inactive (n=29) SLE-active (n=31) Healthy control group (n=31)	PD was defined as the presence of interproximal loss of attachment ≥3 mm and PPD ≥4 mm in at least 2 sites of different tooth, according to CDC/AAP criteria.	PPD, CAL, BOP	SLEDAI (2000). Disease severity is measured according to SLICC/ACR-DI	SLE-I individuals showed increased CAL and worsen periodontal clinical outcomes (8.31 ± 9.438) than SLE-I and healthy controls, but with no significant differences between groups, except for BOP which was significantly reduced in both SLE-A and SLE-I compared to controls.	Altogether, low-grade systemic inflammation that influenced SLE disease activity and severity was correlated to dysbiotic changes of the oral microbiota present in PD.
Mendonca et al. 2019 [255], Brazil **Immunological signatures in saliva of systemic lupus erythematosus patients: influence of periodontal condition**	Case-control	Case group 1 (n=70) SLE patients. (n=70) non-SLE Patients (control group)	CP was defined as ≥2 interproximal sites with CAL ≥3 mm, and ≥2 interproximal sites with PPD ≥4 mm (not on same tooth) or one site with PD ≥5 mm (Eke at al. 2012)	PPD, CAL, BOP and missing teeth	SLEDAI 2000 and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.	Positive correlations were observed between periodontal destruction (PPD, CAL, concomitant sites and missing teeth) and SLE duration (p=0.016, p=0.003, p=0.024 and p=0.006 respectively).	Long-term therapy with corticoids would contribute with periodontal destruction in SLE patients. Moreover, the increased levels of IL-6, IL-17A and IL-33 in saliva of SLE subjects with CP may signal it as possible inflammatory pathways in this process.

Rezaei et al. 2019 [256], Iran **The comparison of visfatin levels of gingival crevicular fluid in systemic lupus erythematosus and chronic periodontitis patients**	Case-control	Total subjects (n=60) Healthy subjects (n=15) control group. CP (n=15) SLE+PD (n=15) SLE+ without PD (n=15) with healthy subjects	CP was diagnosed according to criteria defined by the AAP (greater than 5-mm clinical attachment loss (CAL) in greater than 30% of sites)	PPD, CAL, BOP	Revised ACR (1997). Systemic Lupus Erythematosus Disease Activity Index (SLEDAI 2000)	The frequencies of SLE-CP, SLE-H, H-CP, and H-H groups were 23.63% (n = 13), 27.27% (n = 15), 27.27% (n = 15), and 21.83% (n = 12) respectively. The mean age of the case and control groups was 44.31 (SD = 9.18) and 43.68 (SD = 8.17)	Visfatin levels have correlated positively with all the clinical periodontal parameters and its levels in (L-CP) group are highest in comparative with other groups. Results suggested visfatin has a possible role in association between SLE and PD.

Table 6. *Evidence Table for Case-Control Studies*

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Evidence Table for Cross-Sectional Studies							
1st Author, Year, Country, Title	**Study Design**	**Sample Size**	**Periodontal Criteria**	**Periodontal Parameters**	**SLE Diagnosis**	**Main Findings**	**Published Conclusion**
Al-Mutairi [257] et al, 2015 , Kingdom of Saudi Arabia Periodontal findings in systemic lupus erythematosus patients and healthy controls	Cross-sectional	Cases group (n=25): SLE patients Control group (n=50): healthy subjects	Periodontal assessments consisted of the following: PPD, CAL, PI, BOP and the number of teeth present.	PPD, CAL, BOP, and PI	ACR criteria (1997)	The mean sites with CAL of ≥2 mm was 75.8% in SLE group however, it was 71.9% for the control group (p=0.314). The mean PPD was 2.26 ± 0.47 in group 1 (those who had recent flare-ups) compared with 2 3.09 ± 1.42 mm group 2 (who were stable for more than one year) (p=0.066). The percentage of PPD (≥5 mm) was significantly higher in those who had recent flare-ups compared with those who were stable for more than a year (p=0.032). The PD parameters were also measured in SLE patients with or without arthritis, the mean PPD was 2.48 ± 0.53 mm in SLE patients who had arthritis compared with 2.55 ± 1.06 mm who had none (p=0.371). The percentage of PPD (≥5 mm) was higher in arthritis patients compared with those who had none (p=0.053). The mean CAL (p=0.002) and percentage of CAL ≥2 mm was significantly higher among those with arthritis (p=0.021). In SLE patients, the percentage of sites with ≥5 mm PD was 17.90 ± 39.71 mm in patients with nephritis, whereas it was 9.27 ± 23.14 mm in patients without nephritis.	Periodontal health was not different between SLE patients and healthy controls. However, SLE patients' severity of symptoms and presence of arthritis had a significant relation with PD.
Zhang et al, 2017 [91], China Periodontal disease in Chinese patients with systemic lupus erythematosus	Cross-sectional	Cases group (n=108): SLE patients Control group (n=108): healthy subjects	PI and GI were evaluated according to the SILNESS and LOE, method. PPD, CAL and BOP.	PPD, CAL, GI, PI, and BOP	ACR criteria (1982/1997) and SLE activity by SLEDAI	The periodontal status was significantly worse in SLE patients compared to controls (P<0.001) The prevalence of PD was significantly higher in the SLE group compared with the control group (P<0.001). A positive association between diseases was found (OR=13.9; 95% CI:5.1-38.3, P<0.01).	Chinese SLE patients have an increased prevalence of PD and gingivitis compared to the healthy individuals.
Gofur et al, 2019 [258], Indonesia Periodontitis is associated with disease severity and anti-double stranded DNA antibody and interferon-gamma levels in patients with systemic lupus erythematosus	Cross-sectional	SLE patients (n=61)	Periodontal index was assessed by the method introduced by (Aguilar and Eke at el. 2012)	Periodontal index (Mild. Moderate, Severe PD)	SLEDAI (2000)	SLE severity was evaluated according to the average SLEDAI score (17.70 ± 12.70), and average anti-dsDNA (122.6 ± 81.01 U/mL), and IFN-g (14.64 ± 11.17 pg/mL) levels. There was a significantly positive correlation between PD and SLEDAI score (r = 0.927; p ≤ 0.0001), anti-dsDNA antibody (r = 0.948; p ≤ 0.0001), and IFN-g (r = 0.951; p ≤ 0.0001) levels.	PD was associated with SLEDAI and was a biomarker of immune aging. This biomarker could be a reliable predictor of periodontal condition and prognosis of PD and can also help in selecting the most appropriate treatment strategy for PD in SLE patients.

Table. 7. *Evidence Table for Cross-Sectional Studies*

Evidence Table for Randomized control trial							
1st Author, Year, Country, Title	**Study Design**	**Sample Size**	**Periodontal Criteria**	**Periodontal Parameters**	**SLE Diagnosis**	**Main Findings**	**Published Conclusion**
Fabbri et al, 2014 [173], Brazil Periodontitis treatment improves systemic lupus erythematosus response to immunosuppressive therapy	Randomized controlled trial	Cases group (n=32): SLE and PD patients + immediate periodontal treatment Control group (n=17): SLE and PD + periodontal treatment after 3 months	GI was determined by the percentage of bleeding teeth after periodontal probing. PPD was evaluated by the mean distance from the free gingival margin to the bottom of the pocket. CAL was defined by the mean distance from the CEJ to the bottom of the pocket. (Method by Loe H *et al*. 1967)	GI, PPD, and CAL	ACR criteria (1982) and SLE activity by SLEDAI	SLEDAI and periodontal parameters were determined at entry and after 3 months. Age, female gender, and race were alike among treated and not treated (p>0.05). Treated group had a significant improvement in SLEDAI (5.9±4.2 vs. 3.4±3.3, p=0.04) with a paralleled reduction in GI (40.8± 31.0 vs. 15.2±17.2 %, p<0.01), PPD (1.7±1.8 vs. 1.1±0.3 mm, p<0.01), and CAL (2.5±1.9 vs. 1.7±0.9 mm, p<0.01). In contrast, SLEDAI (6.3±4.3 vs. 6.0±5.5, p=0.40) and PD parameters [GI (p=0.33), PPD (p=0.91), and CAL (p=0.39)] were non-significant in the not treated group.	Periodontal disease treatment might have a beneficial effect in controlling disease activity in SLE patients under immuno-suppressive therapy.

ACR: American college of Rheumatology; AAP: American academy of Periodontology; CP: Chronic Periodontitis; BOP: Bleeding on probing; CAL: Clinical attachment level; DM: Diabetes Mellitus; GI: Gingival Index; PD: Periodontitis; PI: Plaque Index; PPD: Probing pocket depth; SLE: Systemic lupus erythematosus; SLEDAI: Systemic lupus erythematosus Disease activity index; VPI: Visible Plaque Index; HTR: Heart transplant recipient; CEJ: Cemento-enamel junction

Table 8. *Evidence Table for Randomized control trial.*

2.8 Risk of Bias (RoB) and Quality Assessment

The methodological quality of studies included in this review was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies [259]. Studies were scored as low RoB (7-9 stars), moderate RoB (4-6 stars) and high RoB (1-3 stars). Criteria for qualitative assessment comprised the following items: sample selection, comparability, and exposure. Each of the items was assessed and graded (1 or 2 points) according to the suggested criteria. In this analysis, studies with NOS scores of 1–3, 4–6, and 7–9, were defined as of low, intermediate, and high quality, respectively (Table Randomized controlled trial was appraised (Table 9) using the relevant Cochrane tool (ROB 2.0) [260].

1st Author, Year	Selection	Comparability	Exposure	Score (Rate)
Mutlu 1993 (26)	*	*	*	High (3/9)
Novo et al., 1999 (39)	***	*	*	Moderate (5/9)
Meyer et al.2000 (38)	****	*	-	Moderate (5/9)
Kobayashi et al. 2003 (22)	***	*	*	Moderate (5/9)
Kobayashi et al. 2007 (2)	***	*	*	Moderate (5/9)
Wang et al. 2015 (27)	*****	*	-	Moderate (6/9)
Mutairi et al.2015 (25)	***	*	-	Moderate (4/9)
Marques et al. 2016 (23)	**	*	*	Moderate (4/9)
Calderaro et al. 2016 (21)	***	*	*	Moderate (5/9)
Zhang et al. 2017 (3)	***	*	*	Moderate (5/9)
Wu et al. 2017 (29)	**	*	*	Moderate (4/9)
Correa et al.2017 (24)	****	*	*	Moderate (6/9)
Correa et al.2018 (28)	***	*	*	Moderate (5/9)
Pessoa et al. 2019 (41)	***	*	***	Low (7/9)
Mendonca et al. 2019 (42)	****	*	***	Low (8/9)
Rezaei et al. 2019 (43)	****	*	**	Low (7/9)
Gofur et al. 2019 (44)	***	*	**	Moderate (6/9)

Table 9. *Quality assessment of included observational studies according to the Newcastle-Ottawa Scale.*

Study (First author)	Study design	Bias arising from the randomization process	Bias arising from the intended interventions	Bias due to missing outcome data	Bias in measurement of the outcome	Bias in the selection of the reported result	Overall risk of bias
Fabbri et al. 2012 **[173]**	RCT	High	Low	Low	High	High	High

Table 10. *Quality assessment of included Randomized control trial.*

2.9 Statistical analysis

Quantitative analyses were performed and estimates were calculated using a DerSimonian-Laird random-effects model [129] using R statistical software (version 3.4.1, R Studio Team, 2018) as previously described [261]. Mean and standard deviations were estimated by using the formulas introduced by Hozo et al., 2005 [247]. Forest plots provided visualization of estimates and their 95% confidence intervals (CIs). Random-effects meta-analysis and forest plots were produced using 'meta' package [262].

Subgroup meta-analyses according to the periodontal status was conducted for PPD and CAL. Overall, this subgroup approach considered studies of three types: studies with data concerning PD patients (both SLE and control groups); studies with data concerning non-PD studies (both SLE and control groups); and studies that have not report the prevalence of PD (in both SLE and control groups). This last subgroup was named as "PD and non-PD patients" for PPD and CAL analyses (Figure 12 and 13).

Quantity I2 measured the degree of dispersion of effect sizes (ES) estimates and $\chi 2$ test assessed the overall homogeneity statistical significance [263]. All tests were two-tailed, with alpha set at 0.05 except for the homogeneity test whose significance level cut-off was set at 0.10, due to the low power of the $\chi 2$ test in the context of a limited number of eligible studies. Overall ES estimates were reported with 95% confidence intervals (CI).

2.10 Results

2.10.1 Study selection

A total of 1183 studies were identified using the search strategy. After removal of duplicates (n=892) further 870 articles were excluded as did not fulfil the inclusion criteria. Out of the remaining 22 full-length articles that were screened, 4 were further

excluded leaving a final sample of 18 manuscripts deemed appropriate to be included in the qualitative analysis whilst 13 were selected for quantitative analysis (Figure 11). Good agreement between the reviewers was achieved (k-value = 0.947, 95% Confidence Interval [CI]: 0.932-0.962).

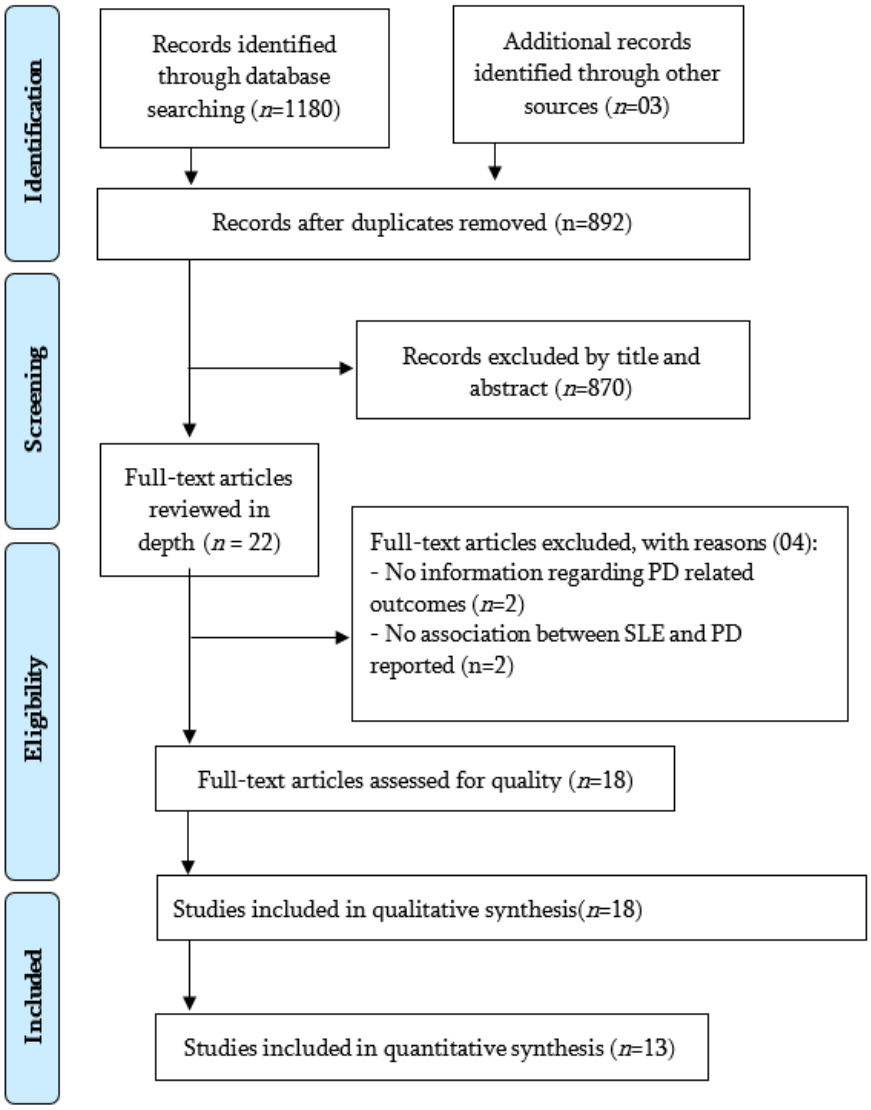


Figure 11. *Prisma flow chart.*

2.11 Assessment Risk of Bias within studies

The majority of the observational studies included in this review presented with a moderate risk of bias (Novo et al. 1999 [8]; Meyer et al. 2000 ; Kobayashi et al. 2003; Kobayashi et al. 2007; Al-Mutairi et al. 2015; Wang et al. 2015; Marques et al. 2016; Calderaro et al. 2017; Corrêa et al. 2017; Wu et al. 2017; Zhang et al. 2017; Corrêa et al. 2018; Gofur et al. 2019), one with high (Mutlu et al. 1993) and three with low risk of bias (Mendonça et al. 2019; Pessoa et al. 2019; Rezaei et al. 2019) (Table 9). Amongst the main factors behind study bias we have identified: definition adequacy, representativeness of the cases, selection and definition of the controls, representativeness of exposed cohort and demonstration that outcome of interest was not present at start of study. One randomized controlled trial was identified and presented with high risk of bias (Table 10) [173].

2.12 Primary Outcome

From a cumulative sample of almost 80,000 individuals, it has been observed that the patients with SLE had greater odds of PD diagnosis when compared with controls without SLE (OR=1.33-95%CI: 1.20-1.48) for both sex (Figure 12), with complete homogeneity (I^2=0.0%). When studies including only female patients were evaluated, the odds of PD were not significantly different between groups (OR= 3.20-95%CI: 0.85-12.02) (figure 13), however this estimate had high heterogeneity (I^2=91.0%, p<0.01).

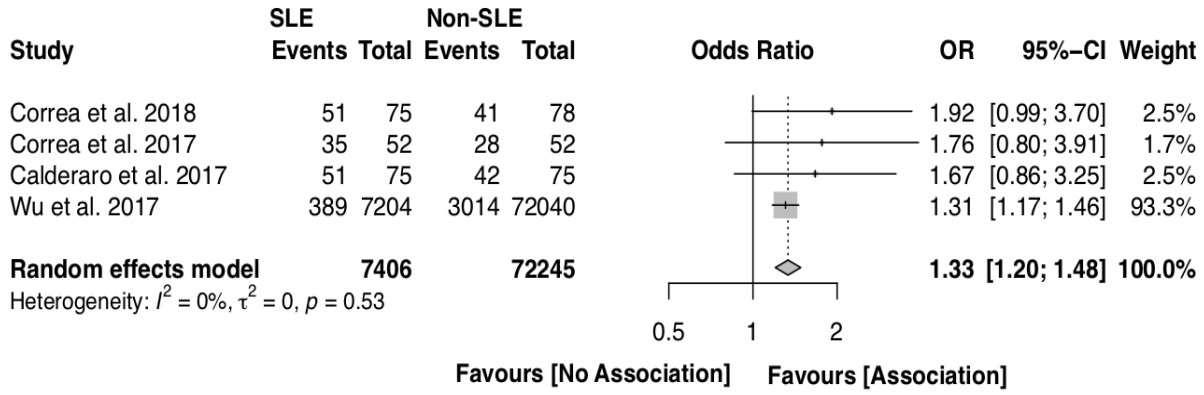


Figure 12. *Summary Forest plot for odds ratio of PD in both sex between SLE and non-SLE patients in cross-sectional and case–control studies. The result tends to favor higher odds for PD in SLE patients (p<0.001). The random effects model was used, and the relative size of the data markers indicates the weight of the sample size from each study. Mean effect size estimates have been calculated with 95% confidence intervals as shown above. Diamond and the vertical dotted line represent the overall pooled estimate.*

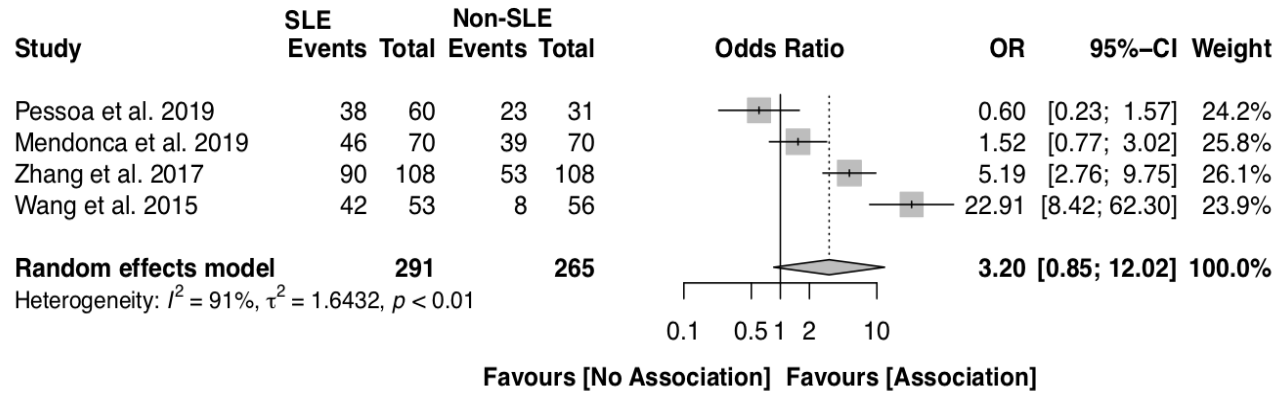


Figure 13. *Summary Forest plot for odds ratio of PD in females SLE and Non-SLE patients in cross-sectional and case–control studies. The result tends to show no significant increase in the incidence of PD in SLE female patients (p= 0.073). The random effects model was used, and the relative size of the data markers indicates the weight of the sample size from each study. Mean effect size estimates have been calculated with 95% confidence intervals as shown above. Diamond and the vertical dotted line represent the overall pooled estimate.*

2.13 Secondary Outcomes

2.13.1 Effect of Systemic Lupus Erythematosus on Dental Parameters

When gingival parameters were compared between patients with and without SLE, no statistically significant differences were noted. A minimal difference in PPD was observed between patients with SLE and controls (SMD of -0.09 mm-95%CI: -0.45-0.27) but with a high level of heterogeneity (I2=90.0%, p<0.01) (Figure 14).

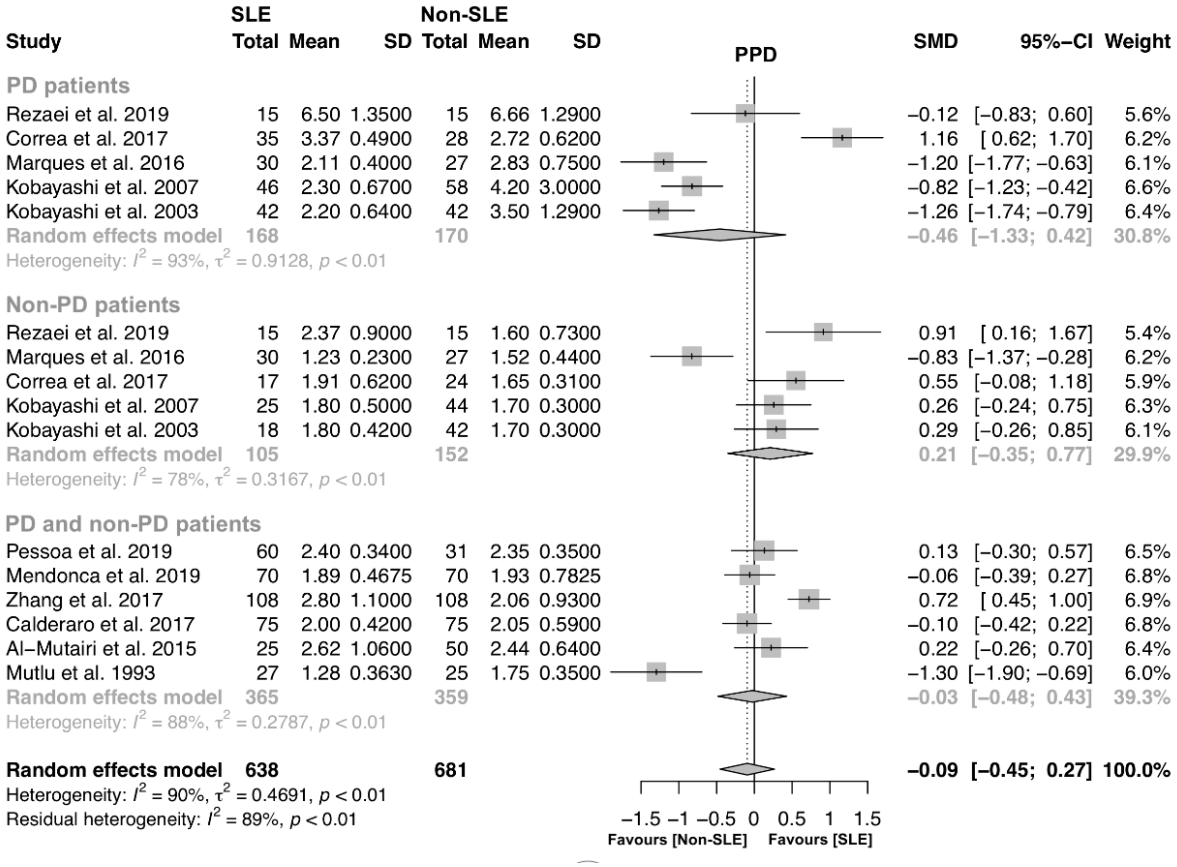


Figure 14. *Summary Forest plot of mean difference in PPD in relation to SLE status in cross-sectional and case–control studies. The results showed no significant changes in PPD in patients with SLE, PD and Non-PD (p=0.611). The random effects model was used, and the relative size of the data markers indicates the weight of the sample size from each study. Mean effect size estimates have been calculated with 95% confidence intervals as shown above. Diamond and the vertical dotted line represent the overall pooled estimate.*

Similarly, no difference in CAL was observed when SLE patients with PD were compared with healthy controls with PD (WMD of 0.05 mm-95%CI: -0.30-0.40), with high level of heterogeneity (I2=89.0%, p<0.01) (Figure 15). For the remaining periodontal parameters, meta-analyses were not possible to perform.

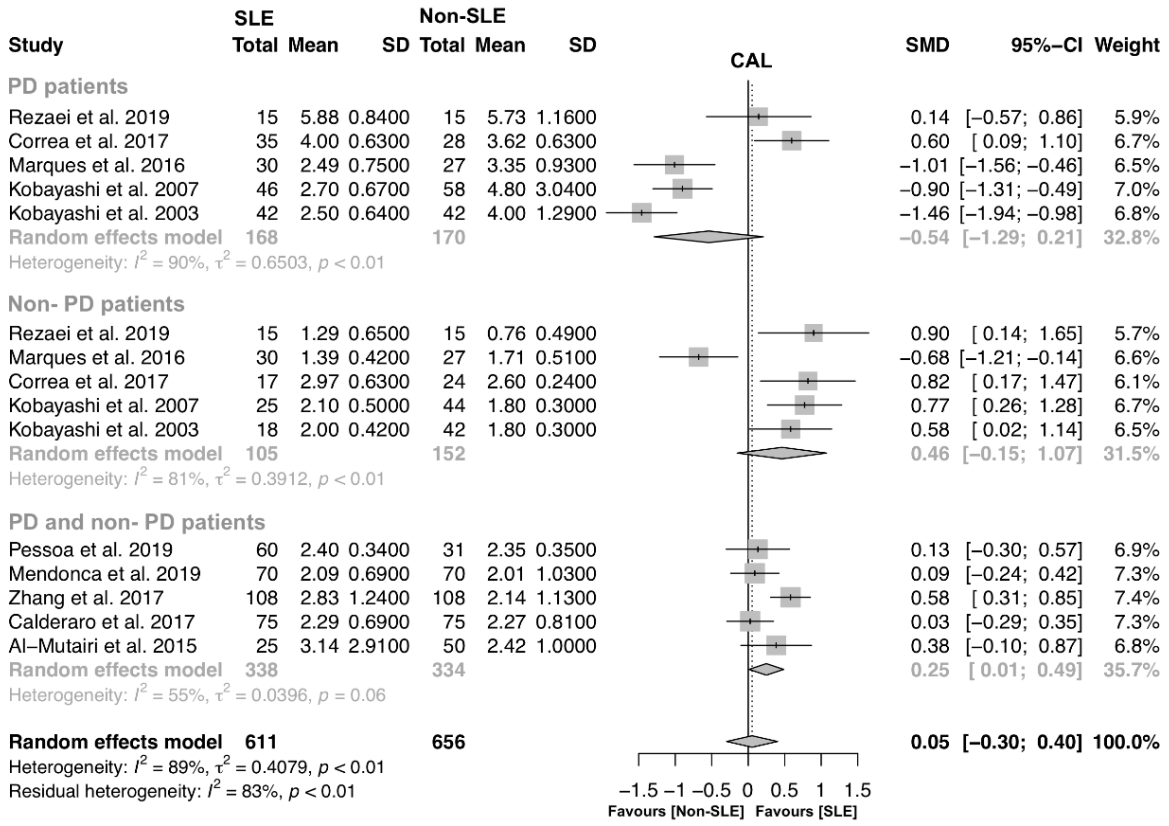


Figure 15. *Summary Forest plot of mean difference in CAL in relation to SLE status in cross-sectional and case–control studies. The results showed no significant changes in CAL in patients with SLE, PD and Non-PD (p=0.766). The random effects model was used, and the relative size of the data markers indicates the weight of the sample size from each study. Mean effect size estimates have been calculated with 95% confidence intervals as shown above. Diamond and the vertical dotted line represent the overall pooled estimate.*

2.13.2 Effect of Periodontitis on SLEDAI scores

Diagnosis of PD was associated with greater SLEDAI scores in patients with SLE (SMD of 0.68-95% CI-I2=72.0%-p=0.03) (Figure 23).

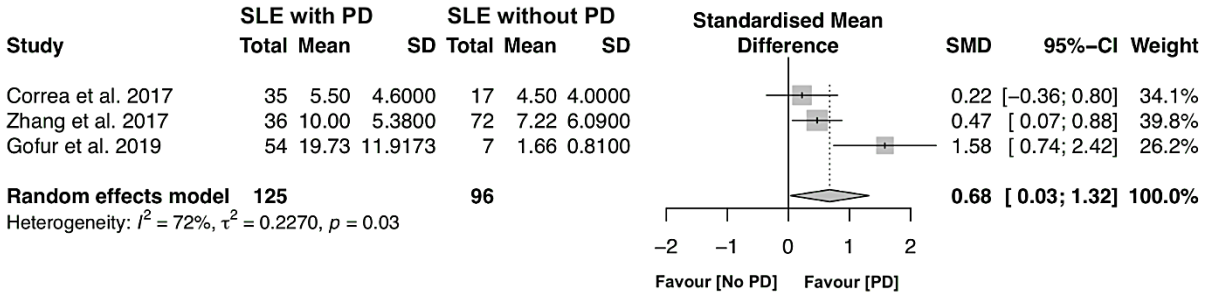


Figure 16. *Summary Forest plot of SLEDAI standardised mean difference in relation to PD status in cross-sectional and case–control studies. . The result tends to favor the increase of SLEDAI score in SLE patients when PD is present (p= 0.039). The random effects model was used, and the relative size of the data markers indicates the weight of the sample size from each study. Mean effect size estimates have been calculated with 95% confidence intervals as shown above. Diamond and the vertical dotted line represent the overall pooled estimate.*

2.14 Discussion

The review suggested that the SLE patients have moderately higher odds of PD. However, the SLE patients did not exhibit worse periodontal parameters when compared with controls. Furthermore, patients suffering with SLE when diagnosed with PD exhibited worsen SLEDAI scores, but limited evidence was found on the beneficial effect of the treatment of PD on SLE outcomes.

A previous systematic review published in 2017 concluded that SLE is associated with a higher risk of PD compared with controls (RR = 1.76-95% CI-1.29-2.41) [7] which support the results of this systematic review. Another systematic review by Zhong et al, in 2020 has confirmed the association between PD and SLE however, the data does not represent the observations of the current studies which has been updated through this review [264].

In contrast, this review gathered additional data from more recent publications which resulted in a reduced estimate of association between the two diseases. Further we investigated the impact of sex differences in the association between PD and SLE. This disease is far more common in women, and this might be related to Oestrogen/Progesterone imbalance and its impact on the host inflammatory response [265, 266]. This could also play a role in the increased susceptibility to PD. Notwithstanding, our meta-analytical results were not able to confirm the link between diagnosis of PD and SLE in females' participants. Further research in understanding the potential mechanisms of sex influence on the association between PD and SLE are needed. The focal point for the interaction of the body with the external environment is the oral cavity, which is consist of different surface types and each is colonized by several species. These species include *bacteria, fungi, protozoa and viruses* [267, 268]. The makeup of the oral microbiome is majorly impacted by the level of the oral hygiene of an individual. A healthy individual with better oral hygiene will have an unpretentious flora which is dominated by the *gram-positive rods* and *cocci* with few *gram-negative cocci*. Whereas individuals with poor oral hygiene will have a more complicated flora dominated by the *an-aerobic gram-negative* bacteria [269].

Regular dental care can prevent periodontal diseases (gingivitis and PD) and benefit the patients who are at a higher risk of developing more serious systemic conditions [270]. The systemic conditions that are impacted by the level of the oral hygiene or condition are atherosclerotic disease, pulmonary disease, kidney disease, pregnancy, osteoporosis, diabetes, and birth weight [271].

Several mechanisms have been proposed to explain the association between auto-immune diseases and PD. SLE is characterized by a loss of self-tolerance, uncontrolled activation of autoreactive T and B cells leading to production of pathogenic autoantibodies, as well as deficits in apoptosis leading to chronic inflammation and damage [90]. SLE is assumed to occur when there is an environmental stimulus that initiates inflammation in a 'genetically primed individual'. In a similar fashion, PD is characterized by a deregulated inflammatory response triggered by a dysbiotic dental biofilm [272].

Further evidence of a link between PD and SLE comes from reports confirming expansion of B-cells and plasma cells in oral lesions and peripheral blood or affected organs [273]. Common genetic predisposition has been suggested as a plausible mechanism of association between PD and SLE, indeed Fcγ receptor polymorphisms have been identified in both diseases (Kobayashi et al. 2003). Further TLR-4 stimulation by bacteria promoted development of SLE in and experimental mouse model (Marques et al. 2016).

There is convincing evidence that infection caused by the pathogenic bacteria may interact with the adaptive and innate immune system, resulting in abnormal production of the autoantibodies, which are the hallmark of many autoimmune diseases, including SLE. Infectious agents including those from the oral/periodontal environment can interfere with the immune system in various ways such as exposure of camouflaged antigens to the immune cells, an altered apoptosis of host cells or molecular mimicry (Fairweather and Rose 2004).. The most studied is the bacterial contribution of *Porphyromonas gingivalis* through mechanisms of substrate-specific and site-specific citrullination (Bingham and Moni 2013). In SLE patients, neutrophils had an increased capacity to form NETosis (neutrophil extracellular traps) that can anchor auto-antigens that may include dsDNA, granular proteins and chromatin (Tsokos 2020).

Wang et al. found that patients with SLE who also harboured *Treponema denticola* and P. gingivalis or combination of both had increased titers of anti-β2-glycoprotein I and anti-cardiolipin antibodies (Wang et al. 2015). Similarly, *Aggregatibacter actinomycetemcomitans* is known to be a potential trigger of autoimmune response in diseases such as RA and SLE (Rutter-Locher et al. 2017). Bagavant et al. identified that the presence of submucosal bacterial infection had impact on SLE disease activity. Anti-dsDNA antibody titre (used as a biomarker in SLE) strongly associated with the presence of *Aggregatibacter actinomycetemcomitans* in patients with PD, suggesting that these bacteria might contribute towards accelerating the anti-dsDNA activity in patients with SLE (Bagavant et al. 2019). A recent study by Sete et al. also identified that patients with juvenile SLE have worse PD and this was associated with altered levels of pro-inflammatory cytokines in gingival crevicular fluid and increased number of *Aggregatibacter actinomycetemcomitans* in the intrasulcular biofilm (Sete et al. 2019).

It has already been proposed that patients with PD might be at increased risk of RA and SLE due to a hyper-inflammatory trait (Bae and Lee 2020). There is however no current consensus regarding periodontal screening of SLE patients. Further research should promote discussion amongst health professional and recognition of the close link between these diseases.

This review observed the potential impact of SLE on clinical periodontal measures and although there was no statistical difference in common measure of periodontal health, it was obvious an opposite trend of gingival inflammation/loss in patients with or without SLE. Therefore, patients with PD and SLE had a tendency of presenting with minimal periodontal inflammation but greater gingival attachment loss. This preliminary finding could be linked to the effect of SLE treatments on periodontal tissues (Sete et al. 2019). SLE medications indeed could mitigate the severity of PD. Further investigations are needed to understand the relative role of immunomodulators in SLE and their impact on periodontal tissues.

The review provided evidence for a direct impact of PD on SLE disease activity as assessed by a worsened SLEDAI score (0.68, p=0.039). Two studies used the classic SLEDAI score (Zhang et al. 2017; Gofur et al. 2019) whilst Correa et al. adopted its updated version (SLEDAI-2K) (Corrêa et al. 2018). The two indices are comparable but not similar. SLEDAI assesses only new features of SLE while the SLEDAI- 2K version also rates the deteriorating symptoms and existing symptoms in some domains. The impact of PD on SLEDAI reported in this review (SMD=0.68) could have limited clinical significance, nevertheless it is plausible to consider that the PD host response could represent an overlooked factor of SLE symptoms exacerbation. Pessoa et al. already hypothesized that the inflammatory response initiated by the oral microbiota (*P. gingivalis*) could influence the severity of SLE.

Treatment of PD may lower systemic inflammation and decrease of systemic inflammation in these patients (Sete et al. 2019). The interventional evidence of a potential benefit of managing PD in SLE is however limited. There is only one randomized controlled trial reporting the effects of periodontal treatment on SLEDAI scores in SLE patients (Fabbri et al. 2014). However, they observed within-group difference after

therapy. Whilst both study groups had similar SLEDAI scores at the start of the study (5.9±4.2 vs. 6.3±4.3, p=0.73), after treatment an improvement of SLEDAI was reported only in the treated group i.e. (5.9±4.2 vs. 3.4±3.3-p=0.04). Furthermore, there is no significant difference observed between groups concerning the disease duration (10.7±6.8 vs. 11.0±6.6, p=0.83).

The effect of potential confounders including immunosuppressive regimens (corticosteroids, and IVCYC), disease duration, and inflammatory markers were observed insignificant and do not influence the risk and severity of PD (Been and Engel 1982; Safkan and Knuuttila 1984; Sooriyamoorthy and Gower 1989; Mutlu et al. 1993). In addition, the use of the oral steroidal therapy was found associated with the plaque and gingival indices in juvenile SLE (Fernandes et al. 2007). These findings are in line with previous evidence supporting a role in treating PD and a significant decrease in DAS28 in RA patients (Al-Katma et al. 2007; Ortiz et al. 2009). PD has been consistently linked to an endothelial dysfunction, it might also be possible that PD in SLE patients could contribute to their raised vascular risk (Sete et al. 2019). Future research should focus on the potential impact of PD and its associated cardio-metabolic derangements in patients with SLE and the potential of intervention trials to explore causality.

There are some limitations in this review that should be highlighted. Firstly, we identified a high level of heterogeneity between the included studies which prevents drawing any definitive conclusions on the impact of PD on SLE. Limited number of studies and patient's evaluation is still far from ideal to formulate valid conclusions on this association. A compelling limitation in our systematic review was the realization of an inconsistent use of PD case definitions within the included studies. This review has however comprehensively appraised all the evidence linking PD and SLE using a robust methodology. Further our aim was to assess the association between the two diseases without focusing only on a unidirectional link (SLE causing PD) but reviewing the potential impact of PD on SLE and its complications/outcomes. There is no doubt that more high-quality evidence should be produced on this topic: better observational studies should be conducted as well as experiments on understanding the exact mechanisms linking PD and SLE and inevitably efforts should be devoted to ascertaining the potential benefit of

promoting periodontal health in patients with SLE to demonstrate systemic health benefits.

2.15 Conclusion

This review suggested that patients with SLE have greater odds of suffering from PD but not necessarily presenting with worse gingival inflammation of patients without SLE. Furthermore, resolution of gingival inflammation could be a novel non-pharmacologic intervention to improve SLE outcomes.

Chapter 3: Is there a bidirectional association between Rheumatoid Arthritis and Periodontitis? A systematic review and meta-analysis

Study II: Is there a bidirectional association between Rheumatoid Arthritis and Periodontitis? A systematic review and meta-analysis

3 Introduction

RA is an autoimmune rheumatic disease characterized by chronic joint inflammation leading to increased morbidity and mortality (1–3) and if not adequately managed, RA causes progressive disability and systemic complications to patients leading to early death. In addition, RA is associated with high cost for society (1,2,4,5).

Prevalence for RA is estimated between 0.5-1.0% cases in the general population (6) while its cause remains unclear; RA prognosis is heavily influenced by how early diagnosis is made and whether the response to treatment is adequate (1,5). Clinicians use a validated Disease Activity Score assessing 28 selected joints (DAS28) to document and monitor disease activity as well as to guide treatment decisions (7). RA patients frequently exhibit increased serum levels of rheumatoid factor and anti-citrullinated peptide/protein antibodies (ACPAs), which are associated with a more severe disease progression and poorer prognosis (8). The process of arginine citrullination (the most common citrullinated peptide implicated in the pathogenesis of RA) is catalysed by the enzyme known as peptidylarginine-deiminase (PAD) and plays a critical *role* in initiating inflammatory responses in RA patients (9,10).

PD is a chronic non-communicable inflammatory disease characterized by progressive destruction of the periodontal apparatus (11). The disease is thought to be caused by an altered host immune response to a dysbiotic dental plaque biofilm (12–17). PD is one of the most prevalent diseases in the world and is undoubtedly an important public health problem (18). The destructive burden of PD has a significant social and economic impact as its worldwide prevalence remains worrisomely high (19–22). Recent evidence suggests that PD is more prevalent in patients with other comorbidities including diabetes

mellitus, cardiovascular diseases and some types of cancers or chronic inflammatory conditions (23–29).

Though triggered by different aetiological factors, RA and PD share common pathophysiological traits. They are both associated with chronic inflammation characterized by similar cytokines profile and leading to localised bone loss (1,5,30–32). Environmental triggers as well as genetic susceptibility are common features of both disorders (1,5,31,33,34).

Recently a promising research hypothesis emerged linking the pathogenesis of RA to PD based on an autoimmune response to citrullinated proteins produced by *Porphyromonas gingivalis* (Pg) PADs (PPADs) (35). *Pg* is considered as a keystone pathogen implicated in the dysbiotic changes of the dental plaque biofilm, but it is not the only potential bacterial trigger linked to RA. Indeed other periodontal pathogens' antibodies responses have been reported in RA including *Prevotella intermedia*, *Prevotella melaninogenica*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* (Aa) (4,32,35,36).

Over the last two decades, cross-sectional evidence has suggested that PD is more prevalent in patients with RA (33,37–40). Longitudinal studies have reported that PD exposure is associated with increased risk of developing RA (41,42). In addition, a recent systematic review was also found evidence supporting a role of RA in the development/risk of PD (43).

Furthermore, PD was associated with obesity and ACPA positivity in RA patients, and it was more prevalent in the 1st degree relatives of RA patients. It is known that 1st degree relatives of RA patients are at a greater risk of developing RA than the general population; therefore in pre-RA individuals related to RA patients the RA the significant periodontal inflammation could contribute to RA pathogenesis (44,45). There is also evidence that C-reactive protein levels, baseline periodontal status and RA activity are all relevant factors for the progression of PD lesions in interproximal sites in early RA individuals, though disease-modifying anti-rheumatic drugs (DMARDs) contributed to the slow-down of PD progression (46).

Lastly some evidence exists on the potential beneficial effect of periodontal treatment on RA disease activity and inflammatory burden (47,48). There is however inconclusive

evidence regarding the potential role of RA associated inflammation on periodontal tissue health (worsen or better gingival condition).

Our aim was therefore to comprehensively review all the evidence linking PD and RA (hypothesizing a bidirectional nature of the association) using a rigorous systematic protocol.

3.1 Materials and Methods

3.1.1 Protocol and registration

This systematic review protocol was approved by all authors and registered in the National Institute for Health Research PROSPERO, International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/PROSPERO, ID Number: CRD42018097324). This review was conducted according to the Cochrane Handbook of Systematic Reviews of Interventions (49) and reported according to the PRISMA guidelines for papers (50) and abstracts (51).

3.1.2 Eligibility criteria

We set a broad overall research question: “Is there a clinical bidirectional association between PD and RA?”, with the following specific questions:

1) “Do RA patients without PD have worse clinical periodontal parameters?

2) “Does RA influence clinical manifestations of PD?

3) “Does PD influence the clinical symptoms of RA?”.

The review followed these respective PI(E)CO statements:

1. Adult patients without PD (Patients – P); RA (Intervention/Exposure – I); No RA (Comparison – C); Probing Pocket Depth (PPD) and Clinical Attachment Loss (CAL) levels (Outcome – O)
2. Adult patients with PD (Patients – P); RA (Intervention/Exposure – I); No RA (Comparison – C); PPD and CAL levels (Outcome – O)
3. Adult patients with RA (Patients – P); PD (Intervention/Exposure – I); No PD (Comparison – C); DAS28, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), DAS28-CRP and/or DAS28-ESR (Outcome – O)

Cross-sectional, case-controls and longitudinal studies were included as well as randomized clinical trials (RCTs).

To address the first research question, studies reporting on the presence of PPD and CAL assessment in RA patients and healthy controls without a previous diagnosis of PD, were included. Studies comparing these two groups of patients without diagnostic confirmation of absence of clinical and radiographic abnormalities in the control group were excluded.

For the second question, studies reporting the presence of PPD and CAL values of RA patients and healthy controls were included.

To address the third question, studies reporting RA outcome measures, such as DAS28, ESR, CRP, DAS28-ESR and DAS28-CRP in RA patients with and without a previous diagnosis of PD were included.

For the second and third questions, studies that have included RA patients and healthy controls without previously knowing their periodontal status following periodontal clinical assessment were excluded due to high risk of reporting bias.

3.1.3 Information sources search

For the selection of studies included in this systematic review, we searched PubMed, Google Scholar and CENTRAL (The Cochrane Central Register of Controlled Trials) for articles published until March 2019. We merged keywords and subject headings in accordance with the thesaurus of each database and applied exploded subject headings. Our PubMed search strategy was based on the following algorithm (MeSH terms): (chronic periodontitis OR periodontitis, chronic OR adult periodontitis OR periodontitis, adult OR periodontal disease OR alveolar bone loss OR attachment loss, periodontal OR periodontal pocket) and (Rheumatoid arthritis OR RA). In addition, grey literature was searched through the OpenGrey portal (http://www.opengrey.eu). The reference lists of included articles and relevant reviews were manually searched. We included both randomized clinical trials (RCTs) and non-RCTs (case-control, cohort studies and cross-sectional) that reported on (A) RA patients with and without associated periodontal diseases and/or (B) patients with PD with and without a concomitant RA diagnosis. There were no restrictions on publication period. Only English language manuscripts were

considered based on the limited amount of evidence and ability to retrieve information from other studies. Authors were contacted when necessary for additional data or clarifications (Table 11).

MeSH Terms		
MEDLINE	**COCHRANE**	**LILACS**
#1 PD **"PD" [MeSH Terms] OR "PD"[All Fields]**	#1 MeSH descriptor: [Rheumatoid Arthritis] explode all trees	Subject descriptor lookups (Periodontal diseases or periodontics) and (Rheumatoid Arthritis) tw:((tw:(PD))) AND (db:("LILACS") AND mj:("Chronic PD" OR "PD" OR "Periodontal Diseases" OR "Periapical PD" OR "Aggressive PD" OR "Gingiva") AND type_of_study:("clinical_trials" OR "case_control" OR "cohort") AND la:("en"))
#2 periodontal disease **"periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields]**	#2 MeSH descriptor: [Rheumatological diseases] explode all trees	tw:(autoimmune diseases) AND (db:("LILACS") AND mj:("Chronic PD" OR "PD" OR "Periodontal Diseases" OR "Periapical PD" OR "Aggressive PD" OR "Gingiva") AND type_of_study:("clinical_trials" OR "case_control" OR "cohort") AND la:("en"))
#3 periodontal abscess **"periodontal abscess"[MeSH Terms] OR ("periodontal"[All Fields] AND "abscess"[All Fields]) OR "periodontal abscess"[All Fields]**	#2 MeSH descriptor: [Rheumatoid Arthritis] explode all trees	
#4 aggressive PD **"aggressive PD"[MeSH Terms] OR ("aggressive"[All Fields] AND "PD"[All Fields]) OR "aggressive PD"[All Fields]**	#3 Any MeSH descriptor in all MeSH products	
#5 chronic PD **"chronic PD"[MeSH Terms] OR ("chronic"[All Fields] AND "PD"[All Fields]) OR "chronic PD"[All Fields]**	#4 MeSH descriptor: [Aggressive PD] explode all trees	
"periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontology"[All Fields]	#5 MeSH descriptor: [Periodontal Abscess] explode all trees	
#7 periodontal attachment loss **"periodontal attachment loss"[MeSH Terms] OR ("periodontal"[All Fields] AND "attachment"[All Fields] AND "loss"[All Fields]) OR "periodontal attachment loss"[All Fields]**	#6 Any MeSH descriptor in all MeSH products	
#8 gum disease **"gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gum"[All Fields] AND "disease"[All Fields]) OR "gum disease"[All Fields]**	#7 MeSH descriptor: [PD] in all MeSH products	
(((((("PD"[MeSH Terms] OR "PD"[All Fields]) OR ("periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields])) OR ("periodontal abscess"[MeSH Terms] OR ("periodontal"[All Fields] AND "abscess"[All Fields]) OR "periodontal abscess"[All Fields])) OR ("chronic PD"[MeSH Terms]	#8 Add #1#2#3	

OR ("chronic"[All Fields] AND "PD"[All Fields]) OR "chronic PD"[All Fields])) OR ("periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontology"[All Fields])) OR ("periodontal attachment loss"[MeSH Terms] OR ("periodontal"[All Fields] AND "attachment"[All Fields] AND "loss"[All Fields]) OR "periodontal attachment loss"[All Fields])) OR ("gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gum"[All Fields] AND "disease"[All Fields]) OR "gum disease"[All Fields])) OR ("aggressive PD"[MeSH Terms] OR ("aggressive"[All Fields] AND "PD"[All Fields]) OR "aggressive PD"[All Fields])		
1- "arthritis, rheumatoid"[MeSH Terms] OR ("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields])		
2- "arthritis"[MeSH Terms] OR "arthritis"[All Fields]		
(("arthritis, rheumatoid"[MeSH Terms] OR ("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields])) OR ("arthritis"[MeSH Terms] OR "arthritis"[All Fields])) OR ("rheumatic diseases"[MeSH Terms] OR ("rheumatic"[All Fields] AND "diseases"[All Fields]) OR "rheumatic diseases"[All Fields] OR ("rheumatic"[All Fields] AND "disease"[All Fields]) OR "rheumatic disease"[All Fields])	#2 Add #1	
3- "joint diseases"[MeSH Terms] OR ("joint"[All Fields] AND "diseases"[All Fields]) OR "joint diseases"[All Fields] OR ("joint"[All Fields] AND "disorders"[All Fields]) OR "joint disorders"[All Fields]		
((((("arthritis, rheumatoid"[MeSH Terms] OR ("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields])) OR ("arthritis"[MeSH Terms] OR "arthritis"[All Fields])) OR ("rheumatic diseases"[MeSH Terms] OR ("rheumatic"[All Fields] AND "diseases"[All Fields]) OR "rheumatic diseases"[All Fields] OR ("rheumatic"[All Fields] AND "disease"[All Fields]) OR "rheumatic disease"[All Fields])) OR ((("arthritis, rheumatoid"[MeSH Terms] OR ("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields])) OR ("arthritis"[MeSH Terms] OR "arthritis"[All Fields])) OR ("rheumatic diseases"[MeSH Terms] OR ("rheumatic"[All Fields] AND "diseases"[All Fields]) OR "rheumatic diseases"[All Fields] OR ("rheumatic"[All Fields] AND "disease"[All Fields]) OR "rheumatic disease"[All Fields]))) OR ("joint diseases"[MeSH Terms] OR ("joint"[All Fields] AND "diseases"[All Fields]) OR "joint diseases"[All Fields] OR ("joint"[All Fields] AND "disorders"[All Fields]) OR "joint disorders"[All Fields])	#3 Add #1#2#	
((((("gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gingival"[All Fields] AND "disease"[All Fields]) OR "gingival disease"[All Fields]) OR ("gingival diseases"[MeSH Terms] OR ("gingival"[All Fields] AND "diseases"[All Fields]) OR "gingival diseases"[All Fields] OR ("gum"[All Fields] AND "disease"[All Fields]) OR "gum disease"[All Fields])) OR ("periodontics"[MeSH Terms] OR "periodontics"[All Fields] OR "periodontology"[All Fields])) OR (("periodontitis"[MeSH Terms] OR "periodontitis"[All Fields]) OR ("periodontal diseases"[MeSH Terms] OR ("periodontal"[All Fields] AND "diseases"[All Fields]) OR "periodontal diseases"[All Fields] OR ("periodontal"[All Fields] AND "disease"[All Fields]) OR "periodontal disease"[All Fields]))) AND (((((("arthritis, rheumatoid"[MeSH Terms] OR ("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields])) OR ("arthritis"[MeSH Terms] OR "arthritis"[All Fields])) OR ("rheumatic diseases"[MeSH Terms] OR ("rheumatic"[All Fields] AND "diseases"[All Fields]) OR "rheumatic diseases"[All Fields] OR ("rheumatic"[All Fields] AND "disease"[All Fields]) OR "rheumatic disease"[All Fields])) OR ((("arthritis, rheumatoid"[MeSH Terms] OR		

("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields])) OR ("arthritis"[MeSH Terms] OR "arthritis"[All Fields])) OR ("rheumatic diseases"[MeSH Terms] OR ("rheumatic"[All Fields] AND "diseases"[All Fields]) OR "rheumatic diseases"[All Fields] OR ("rheumatic"[All Fields] AND "disease"[All Fields]) OR "rheumatic disease"[All Fields]))) OR ("joint diseases"[MeSH Terms] OR ("joint"[All Fields] AND "diseases"[All Fields]) OR "joint diseases"[All Fields] OR ("joint"[All Fields] AND "disorders"[All Fields]) OR "joint disorders"[All Fields]))		

Table 11. *MeSH Terms using for the systematic review and metanalyses*

All publication years till April 2018 were included. The bibliographic references were hand searched through the reference list of the identified papers and previous reviews. Online hand searching of issues within the past 10 years of some periodontal, RA journals was also performed (Table 12).

Periodontal and Medical Journals for Hand-Searching
➢ **Periodontal Journals**
-Journal of Clinical Periodontology
-Journal of Periodontal Research
-Journal of Periodontology
-Journal of Dental Research
-Periodontology 2000
➢ **Rheumatology Journals**
-Annals of Rheumatic diseases
-Arthritis and Rheumatology
-Rheumatology
-Journal of Rheumatology
-Nature review of Rheumatology

Table 12. *Periodontal and Medical Journals for Hand-Searching*

3.1.4 Study selection

Study selection was initially conducted by two authors (SBH and JB), who screened the titles and/or abstracts of the retrieved studies. The final selection of studies was independently performed by three authors (SBH, JB and VM) who reviewed the full text of the selected papers based on the inclusion criteria mentioned above. Any disagreements were resolved by discussion.

3.1.5 Data extraction process and data items

A predefined table was used to conduct data extraction. The extracted data included: the first author's name, publication year, country of origin of the research, study design, study population inclusion/exclusion criteria, number of cases and participants, gender, mean age in years, periodontal diagnostic criteria, and RA diagnosis based on validated classification criteria. Clinical periodontal measures included: PPD, plaque index (PI), missing teeth, bleeding on probing (BOP), and CAL. To assess RA outcome measures the following data were extracted: Disease Activity Score 28 (DAS28), Erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP). All data were independently extracted by three reviewers (SBH, VM and JB) who reached a consensus on all the aspects.

3.1.6 Risk of bias in individual studies

The Cochrane Collaboration's tool was used to assess the risk of bias of randomized clinical trials (52). Case-control and cohort studies were appraised using the Newcastle-Ottawa (NOS) Scale by two authors (SBH and SAZ). Due to the lack of established standard criteria, authors subjectively considered studies achieving 7 to 9 stars on the scale to be of high quality, studies with 5 to 6 stars of medium quality whilst studies with less than 5 stars were deemed of low methodological quality. Disagreements between reviewing authors over the risk of bias were resolved by discussion (Table 14).

3.1.7 Summary Measures & Synthesis of results

Quantitative analyses were performed with studies of similar design. Estimates were calculated in R version 3.4.1 (R Studio Team 2018) using a DerSimonian-Laird random-effects model (53), as previously described (54). Forest plots were produced to visualise estimates and their 95% confidence intervals (CIs). All random-effects meta-analysis and forest plots were performed using 'meta' package (54). Quantity I^2 was measured to assess the degree of dispersion of effect sizes (ES) estimates and the overall homogeneity statistical significance was calculated through the χ^2 test (Higgins et al. 2003). All tests were two-tailed, with alpha set at 0.05 except for the homogeneity test whose significance level cut-off was set at 0.10 due to the low power of the χ^2 test in the context of a limited number of eligible studies. Publication bias analysis was planned to be performed if, at least, 10 or more studies were included (49). Overall ES estimates were reported with 95% confidence intervals (CI).

3.2 Results

3.2.1 Study selection

The search strategy identified 2159 potentially relevant publications. After the exclusion of duplicates (668), 1491 papers were analysed further. 68 articles out of 1491 have fulfilled the inclusion criteria (1424 articles were excluded). Out of these 68 articles which were subjected to full paper review eligibility, 60 articles were excluded as they did not address the research questions, while 8 studies were included in the final analysis of the association between PD and RA and vice versa. After quality assessment, 2 studies were excluded from further analyses due to high risk of bias (55,56), resulting in a final number of 6 observational studies (Figure 17). These studies were characterised by heterogeneity regarding the association between the two diseases and various outcome measures reporting. All studies included information about clinical outcomes and biomarkers for PD and RA, as well as disease treatment and duration.

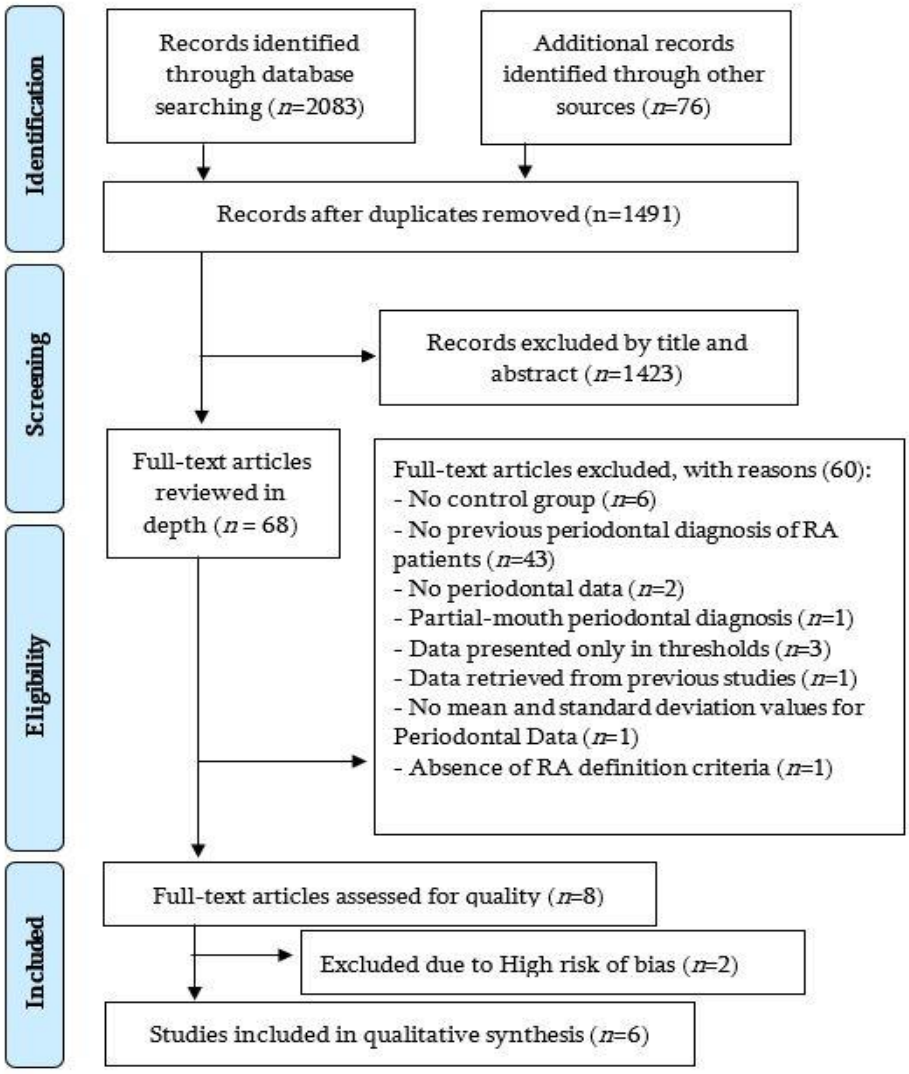


Figure 17. *PRISMA flow-chart representing the results of the workflow to identify eligible studies.*

3.2.2 Study characteristics

We identified 6 case-control studies from four different countries, across Europe and Asia. These studies were published between 2012 and 2019 (Table 13). The sample sizes ranged from 36 (57) to 566 participants (58) per study. Following bias risk assessment, a total of 866 participants were included in this review, comprising 271 RA patients with PD, 68 RA patients without PD, 337 patients with PD and 190 healthy controls.

Authors (Year) (Country)	Number of subjects	Number of subjects per group (Male/Female)				Age (Mean ± SD)				RA Diagnostic Criteria	RA Clinical Characteristics	CP Diagnostic Criteria	Periodontal Clinical Characteristics	CAL (Mean [SD]) (mm)				PD (Mean [SD]) (mm)				DAS-28 (Mean [SD])	
		RA-CP	RA	CP	H	RA-CP	RA	CP	H					RA-CP	RA	CP	H	RA-CP	RA	CP	H	RA-CP	RA
Esen et al. (2012) (Turkey)	80	20 (3/17)	20 (1/19)	20 (4/16)	20 (4/16)	46.3 ± 11.7	44.4 ± 15.5	42.85 ± 9.6	40.05 ± 9.8	ARA 1987 revised criteria (1988)	DAS28	Teeth with CAL ≥4 mm and PD ≥5 mm.	PI, GI, BOP, PD, CAL	5.0 (0.2288)	0.0173 (0.0033)	6.33 (0.3538)	0.015 (0.0028)	5.17 (0.125)	1.670 (0.0825)	6.17 (0.2925)	1.915 (0.165)	3.60 (1.01)	3.15 (1.16)
Sezer et al. (2013) (Turkey)	80	20 (4/16)	20 (2/18)	20 (6/14)	20 (6/14)	44.45 ± 9.52	43.75 ± 11.06	45.50 ± 7.50	40.75 ± 10.26	ARA 1987 revised criteria (1988)	DAS28, TAS, TOS, OSI, ARE, CRL, SH, PON	Flemmig (1999)	PI, PD, CAL, BOP	3.62 (0.58)	0.32 (0.29)	3.69 (0.49)	0.32 (0.23)	3.28 (0.52)	2.06 (0.90)	3.42 (0.43)	2.18 (0.90)	5.75 (1.45)	5.24 (1.74)
Yokoyama et al. (2014) (Japan)	40	10 (1/9)	10 (1/9)	10 (1/9)	10 (3/7)	60.5 ± 9.6	47.9 ± 14.2	56.5 ± 8.6	56.1 ± 11.9	ARA 1987 revised criteria (1988)	DAS28, CRP, RF	AAP / Armitage (1999)	PI, PD, CAL, BOP	3.3 (0.8)	2.4 (0.1)	3.7 (0.8)	2.1 (0.3)	3.1 (0.7)	2.4 (0.1)	3.4 (0.8)	2.1 (0.4)	NA	NA
Kobayashi et al. (2018) (Japan)	566	185 (38/147)	NS	251 (104/147)	130 (38/92)	59.4 ± 11.6	NA	63.9 ± 9.9	57.1 ± 11.4	ACR / EULAR (2010)	DAS28, CRP, IgG, CCP	CDC / AAP (Eke et al. 2012)	PI, PD, CAL, BOP	2.9 (0.6)	NA	3.6 (1.2)	2.3 (0.4)	2.8 (0.5)	NA	2.8 (0.9)	2.1 (0.3)	NA	NA
Zhao et al. (2018) (P.R. China)	64	18 (4/14)	18 (3/15)	18 (4/14)	10 (2/8)	42.82 ± 11.20	43.62 ± 12.84	44.80 ± 9.53	42.23 ± 14.04	ARA 1987 revised criteria (1988)	DAS28, ESR, CRP, ACPA	Inspection, Periodontal probing and X-rays	PD, GI, PI, BOP	NA	NA	NA	NA	3.28 (0.08)	2.20 (0.05)	3.22 (0.08)	2.21 (0.08)	4.60 (0.96)	3.40 (1.20)
Cosgarea et al. (2019) (Romania)	36	18 (4/14)	NA	18 (8/10)	NA	51.61 ± 11.04	NA	43.55 ± 10.56	NA	ARA 1987 revised criteria (1988)	DAS28, ESR, CRP, and RF.	AAP / Armitage (1999)	PD, CAL, BOP	4.28 (0.52)	NA	4.22 (0.42)	NA	2.83 (0.21)	NA	3.40 (0.24)	NA	NA	NA

Table 13. Evidence table for the included studies

DAS28 - Disease Activity Score 28; ERS - Erythrocyte sedimentation rate; CRP - C-reactive protein; ACPA - anti-cyclic citrulline peptide antibody; RF - rheumatoid factor; IgG - immunoglobulin G; CCP - citrullinated peptide; TAS - total antioxidant status; TOS - total oxidant status; OSI - oxidative stress index; ARE - arylesterase; SH - sulfhydryl; CRL - ceruloplasmin; PON - paraoxonase; PD - pocket depht; CAL - clinical attachment loss; BOP - bleeding on probing; PI - plaque index; GI - gingival index.

3.2.3 Assessment of Risk of bias within studies

NOS Scale bias assessment was used for the case-control studies included in this systematic review (Table 14). Two studies were excluded because they have not met the predefined criteria or minimally acceptable risk of bias (55,56). The remaining studies which were rated as high quality studies presented adequate case definition for RA, clear definition of controls, and the same method of ascertainment for cases and controls for both RA and PD (n = 6, 100%) (57–62). Furthermore, most studies were characterised by representativeness of RA patients (n = 5, 83.3%), correct selection of controls (n = 4, 66.7%), appropriate comparability of cases and controls on the basis of the design or analysis and for additional factors (n = 5, 83.3%) and equal non-response rate (n = 5, 83.3%). Finally, two studies had unblinded periodontal diagnosis to case/control status (n = 2, 33.3%).

AUTHOR, YEAR	**SELECTION**				**COMPARABILITY**	**EXPOSURE**			**FINAL SCORE**
	Is the case definition adequate?	**Representativeness of the cases?**	**Selection of controls?**	**Definition of controls?**	**Comparability of cases and controls of design or analysis?**	**Ascertainment of exposure?**	**Same method of ascertainment for cases and controls**	**Non-response rate?**	
Esen et al. (2012)	A	A	B	A	A/B	C	A	A	☆☆☆☆☆☆☆
Sezer et al. (2013)	A	A	A	A	A/B	A	A	C	☆☆☆☆☆☆☆☆
Yokoyama et al. (2014)	A	A	A	A	A/B	A	A	A	☆☆☆☆☆☆☆☆
Mittal et al (2015)	A	B	C	B	A/B	C	A	A	☆☆☆☆☆
Gittaboyina et al (2017)	A	B	C	B	A	C	A	A	☆☆☆☆
Kobayashi et al. (2018)	A	A	A	A	A	B	A	A	☆☆☆☆☆☆☆☆
Zhao et al. (2018)	A	B	A	A	A/B	A	A	A	☆☆☆☆☆☆☆☆
Cosgarea et al. (2019)	A	A	C	A	A/B	C	A	A	☆☆☆☆☆☆☆

Table 14. *Case-control studies bias assessment using the Newcastle-Ottawa Scale (NOS).*

3.3 Synthesis of results

3.3.1 Rheumatoid Arthritis effects on periodontal tissues

Five studies which investigated the effect of RA on the periodontal tissues of patients without clinical diagnosis of PD were included. Only four clinical studies involved CAL assessments (59–61) and five studies specified PPD values (58–62). In total, 100 and 128 individuals were investigated for CAL and PPD levels, respectively. Although not statistically significant, the overall results suggested that RA is associated with increased CAL and PPD levels in patients with no previous diagnosis of PD. A high degree of study heterogeneity was observed in both analyses.

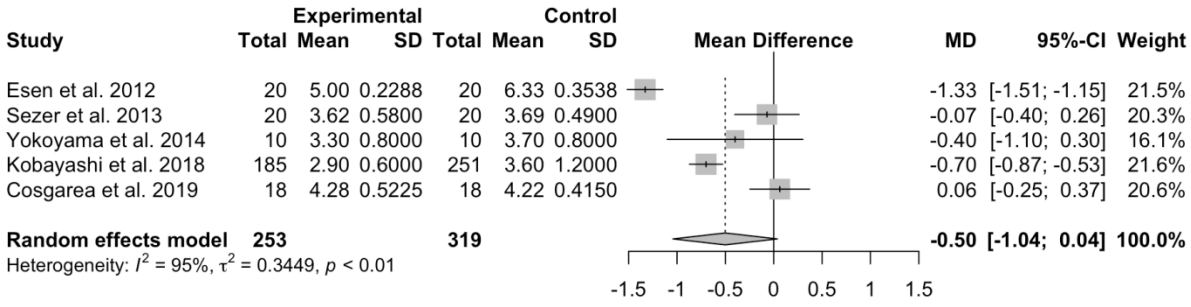


Figure 18. *Forest plot of studies evaluating CAL levels in PD patients with and without RA. Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. The size of the squares is proportionate to the study sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.*

3.3.2 Rheumatoid Arthritis effect on Periodontitis

Six studies including CAL and PPD measurements were selected to investigate the effect of RA on PD clinical characteristics (57–62). Five studies included CAL measures (57–61), while all six had PPD assessments. No statistically significant association between CAL and PPD levels in patients with and without RA were found, and there was a high heterogeneity among the studies included (Figure 18 and Figure 19).

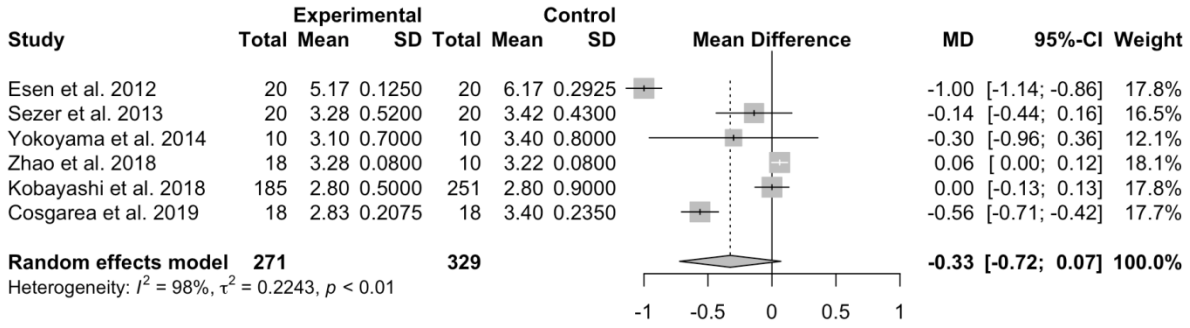


Figure 19. *Forest plot of studies evaluating PPD levels in PD patients with and without RA. Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.*

3.3.3 Periodontitis effect on Rheumatoid Arthritis

Three studies only were included in this analysis (59,60,62), comprising 116 RA patients (58 with and 58 without PD) which had their DAS28 scores calculated. The analysis confirmed an association between PD and increased RA disease activity, with an average of 0.74 DAS28 score points greater in PD compared to non-PD patients ($p < 0.001$) (MD [95% CI]: 0.74 [0.25–1.24]) (Figure 20). The study heterogeneity was low, revealing a high consistency among the included studies. No other quantitative analysis on other RA outcome measures, such as DAS28-CRP, ESR or CRP was performed due to the lack of data reported.

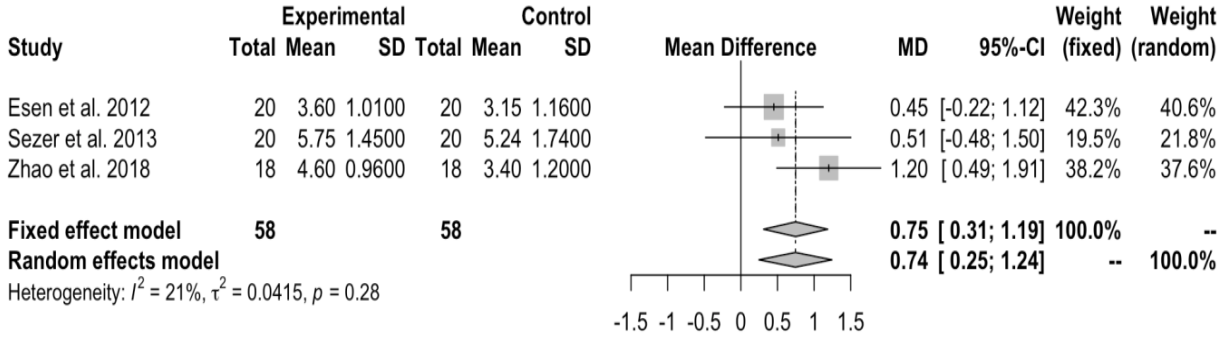


Figure 20. *Forest plot of mean difference in DAS28 scores in RA patients with and without PD. Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.*

3.4 Discussion

3.4.1 Summary of Main Findings and Quality of the Evidence

This review concluded that RA has no significant effect on periodontal tissues. Our results showed that having RA might not worsen clinical periodontal parameters (PPD and CAL) in RA patients with PD compared to PD controls. On the other hand, PD diagnosis was associated with worse RA disease activity.

These results were in agreement with previous studies reporting that RA patients with PD presented with more missing teeth and higher levels of CAL, ESR, CRP and moderate-high RA activity (63,64). Moreover, we appreciate that RA disease duration is likely to have a significant impact on the PD- related outcome measures, and since our analysis has been derived from studies including established RA patients, the results cannot be generalised. It has already been shown that pre-RA cases present with higher periodontal inflammation involvement than established RA patients (44).

The mechanisms by which PD influences RA remain to be clarified, although it is proven that this can be partly mediated by the oral microbiota, for instance *P. gingivalis* and *A.*

actinomycetemcomitans (2,5), since RA patients express higher antibody response to *Pg* (65) and *Aa* (32,36) and can trigger the autoimmunity in RA.

Lately, evidence-based reviews have showed moderate evidence that RA patients have increased risk of developing PD (43,66) and more missing teeth and higher levels of CAL (63) compared to general population In addition, positive associations between various RA biomarkers and PD, such as interleukin-21, resistin and adipokines have been established (67–69). Also, it has been confirmed that nonsurgical periodontal treatment has beneficial effects on RA periodontal patients by improving clinical and inflammatory parameters (47,48).

Although there is lack of data regarding the impact of RA treatment on the PD risk and rate of progression, the most used biologic treatment in RA which targets TNF-α has been shown to reduce the local production of inflammatory cytokines and periodontal inflammation in RA patients with PD (46,64,70). It is therefore possible to speculate that conventional and biologic DMARDs, nonsteroidal anti-inflammatory drugs (NSAIDs), and corticoids which are used for RA treatment could potentially have an indirect beneficial effect on controlling PD inflammation at the gingival level (71,72). However, the results of these studies are contradictory since RA therapies has been reported to have both significant (71) and non-significant adjunctive effects to PD treatment in RA patients (72). Only two studies included in this systematic review have described the type of RA medication used (57,61); however, they have not explored the impact of medication on PD related inflammation. Because of the lack of relevant literature data, investigating the impact of RA medication on PD outcomes has been beyond the scope of our systematic analysis. Interestingly, a recent study has shown that the presence of periodontal inflammation in patients with RA was associated with poorer response to anti TNF-α therapy (73).

Therefore, further well-designed longitudinal trials are required to allow definitive conclusions regarding RA-PD bilateral relationship and potential therapies with shared benefit. In particular, RA patient stratification based on background medication, and degree of periodontal inflammation, as well as larger sample size and longer follow-up are likely to enable a better characterisation of the relationship between PD and RA.

The nonsurgical periodontal treatment (NSPT) efficacy in RA patients with PD has not been investigated by this systematic review, since recent literature found evidence that NSPT improved RA associated parameters, such as DAS28, ESR and inflammatory markers levels (47,48). Nevertheless, these studies had very small sample sizes, hence precluding definitive conclusions.

Overall, the results of this systematic review did not support a bidirectional link between RA and PD but rather point towards a negative impact of PD on RA disease activity. However, we cannot definitively exclude a bidirectional association between both diseases because the studies included in our meta-analysis were associated with high heterogeneity and methodologic variability.

3.4.2 Potential Limitations and Strengths

There were some limitations we wish to highlight to the reader. Firstly, we identified a high level of heterogeneity between the included studies which precluded definitive conclusions on the effect of RA on healthy and unhealthy periodontium. This could be due to the large variability of the periodontal diagnostic criteria used in the included studies. Indeed, while three studies (57,58,61) used recognized at the time case definitions the remaining three used other criteria. Studies included in our meta-analyses assessed predominantly female RA patients, as this reflects the natural prevalence of the disease which is two-to-threefold more likely to affect women than men (74). Male gender was associated however with greater prevalence of destructive periodontal disease (75) and, eventually, may lead to the worsening of RA disease activity and increased morbidity overall. In the future, gender-related risk factors should be considered in RA periodontal patients. In addition, we did not investigate the efficacy of RA medication on PD severity or the impact of PD severity on response to RA treatment. Throughout the review process, several articles were excluded because they compared RA patients with healthy controls without a previous periodontal diagnosis. Future investigations should define the PD status of RA and healthy participants.

This is the first systematic review that reported pooled estimates for clinical parameters linking RA and PD, supported by a thorough literature search, meticulous a priori defined

protocol, and following recommended guidelines for data reporting. Also, all studies included in our analyses were carried out in hospital settings and, considering the relative low prevalence of RA in general population and the fact that RA patients are followed up in hospital rheumatology departments (2), we concluded that our results could be representative of a large group of patients. Importantly, the apparent effect of PD on RA activity assessed by a validated outcome measure (DAS28 score) was remarkable. The pooled estimate of DAS28 score was derived from three studies which had very low heterogeneity (59,60,62), despite using different periodontal diagnostic criteria.

3.5 Conclusion

This systematic review concluded that whilst there is moderate evidence supporting that PD is associated with worse RA disease activity, the contrary relationship (RA worsening PD status) could not be confirmed based on the available evidence.

Periodontal tissues/PD status of patients with RA should be monitored on a regular basis. Rheumatologists and dental professionals should collaborate and aim at improving the clinical outcomes of their patients. In the future, more efforts are needed to comprehensively assess the association between RA and PD, by taking into consideration additional RA clinical characteristics, and to clarify the effect of RA on PD in studies with better methodology and broader clinical and laboratory biomarkers.

Chapter 4: Plausible Links Between Periodontitis and Rheumatological Diseases and an Oral Health Self-Reported Questionnaire based study

Study III: Plausible Links Between Periodontitis and Rheumatological Diseases and an Oral Health Self-Reported Questionnaire based study

4 Introduction

PD is one of the most prevalent oral diseases affecting millions of people worldwide [274]. It is an inflammatory disease characterized by a progressive gingival reaction to a dysbiotic dental biofilm eventually leading to the destruction of all periodontal tissues.

Over the past 3 decades, evidence has confirmed that PD is not confined just to the oral cavity, but it is linked to common co-morbidities and systemic health outcomes [275, 276]. A proposed pathological mechanism suggests that ulceration and inflammation of the subgingival pocket provides the basis for systemic dissemination of bacteria or their end-products and dislocate at other distant sites [277]. Bacterial by-products including lipopolysaccharides have been identified in different organs and may interfere with the existing systemic conditions [275]. Traditional risk factors for PD include age, smoking/tobacco use, genetics, stress, low socio-economic factors, diabetes and recently obesity [71]. It is difficult for patients to report early symptoms, as PD evolves as a silent disease for a long period of time, before signs can be noticed [278]. From a reversible state of gingival inflammation (gingivitis), self-perpetuating chronic inflammation could translate into alveolar bone loss resulting in gingival recession and teeth mobility, which are commonly noted in advanced stages of the disease [279].

RA is a chronic inflammatory condition characterized by joint inflammation, which in majority of cases is symptomatic from the disease onset. The complex autoimmune aetiology of this disease is yet to be clarified [251]. RA can affect hands, elbows, shoulders, neck, hips, knees, ankles, feet, and temporomandibular joints. The chronic inflammatory process associated with RA, if left untreated leads to bone erosions and

cartilage destruction involving both small and large joints but it can also be linked to systemic manifestations [236].

SLE is an autoimmune, chronic, and inflammatory disease that can affect any organ and system [280]. SLE is a disease with heterogenous presentation and in selected cases could lead to life-threatening complications. Because of the unpredictability of progression of SLE, patients need regular monitoring as to prevent or treat exacerbations of the disease in a timely manner and prevent irreversible damage to organs such as lung, kidney, heart, or nervous system [173].

Increasing evidence suggest a potential association between systemic rheumatic diseases and PD. Only a limited number of trials have comprehensively examined PD and its extent in patients with rheumatic diseases hence the available evidence is inconclusive. For instance, Rhodus et al. reported the prevalence of PD in patients with SLE 93.8% [7, 236, 281] whilst in a small Japanese study it has been reported that the prevalence of PD in SLE is 30% as compared to the general population [90]. In addition, the reported prevalence for RA is of 3.95% as compared to 1% in general population [236, 238].

A closer link between these diseases is further supported by preliminary evidences suggesting that periodontal treatment could improve markers of SLE disease activity [173] as well as RA [282]. Immune reactions to common periodontal pathogens like *Aggregatibacter actinomycetemcomitans* (*Aa*) [214] and *Porphyromonas gingivalis* (*Pg*) [283] has opened new avenues of research into possible cross-reactive responses which could suggest PD as a possible trigger of rheumatic diseases.

This study analysis was aimed at describing critical factors contributing to PD/RA and SLE co-existence in some patients using a self-reported Oral Health Questionnaire based study.

4.1 Methods

4.1.1 Study Population

To expand the knowledge on the plausibility links between PD and Rheumatological diseases including SLE and RA, an observational study at the UCLH Rheumatology Department was conducted.

174 RA patients, 210 SLE patients and 14 with both SLE and RA were enrolled and agreed to complete the Self-reported Oral Health questionnaire. The self-reported oral health questionnaire had eight self-report items that were included as a part of the US Centres for CDC/AAP (Disease Control and Prevention Periodontal Disease Surveillance Project, in collaboration with the American Academy of Periodontology) to asses periodontal measures (Appendix 3) [284]. All the participants were adults (above 18-years). Symptoms suggestive of PD were reported by 171 patients in total (45.6 %). Out of the recruited subjects 79 (45.4%) patients with RA and 96 (45.7%) patients with SLE reported symptoms/signs of PD shown in figure 21. In addition, their data findings of other multiple factors were also observed that include Age, Gender, Smoking, Diabetes, Hypertension, Hypercholesterolemia, Statin use, Myocardial Infarction, Stroke, Peripheral vasculopathy, Angina. Blood samples were taken in routine examination to observe the CRP and ESR levels in the participants.

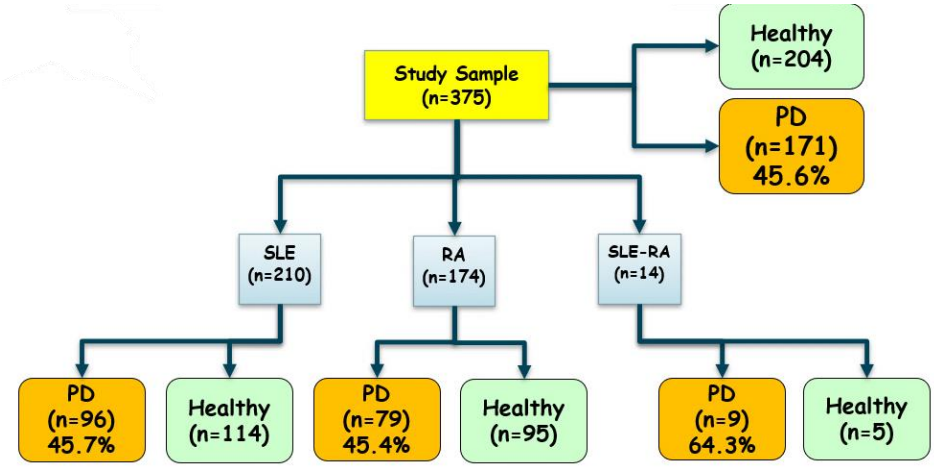


Figure 21. *Diagrammatic representation of the study sample size which shows the total of 375 patients including 204 healthy, 171 with PD that are further divided into patients with SLE (n=210) out of which (n=96) were healthy controls with SLE and (n=114) were PD patients with SLE. Similarly, (n=174) RA patients that include (n=95) healthy controls*

with RA and (n=79) with RA and PD patients. Patients with RA/SLE and PD (n=14) include (n=5) healthy controls and (n=9) with PD.

4.2 Statistical Analysis of the data findings

Data was analysed using SPSS (17.0, Inc., Chicago, IL, USA). Descriptive statistical analyses including means and SD (standard deviation) was processed and evaluated through the t-test, chi-square and fisher's (exact test) using $p<0.05$ as statistically significant [285].

4.3 Results

Recruited patients with PD were more prevalent when participants presented with current smoking (62.5%), hypercholesterolemia (68.4%), hypertension (58.7), statins (69.8 %), peripheral vasculopathy (66.7%) and angina (70%) shown in table 15.

Characteristic table for all patients (n=375)				
Variable	**All**	**Healthy**	**PD**	**p-value**
Age, years	**47.6±16.5**	**45.4±16.8**	**50.1±15.8**	**0.006**
Gender, Female (%)	**324 (86.4)**	**178 (54.9)**	**146 (45.1)**	**0.651**
Current Smoking, n(%)	**40 (10.7)**	**15 (37.5)**	**25 (62.5)**	**0.029**
Diabetes, n(%)	**26 (7)**	**9 (34.6)**	**17 (65.4)**	**0.042**
Hypertension, n(%)	**104 (27.9)**	**43 (41.3)**	**61 (58.7)**	**0.002**
Hypercholesterolemia, n(%)	**57 (15.3)**	**18 (31.6)**	**39 (68.4)**	**<0.0001**
Statin use, n(%)	**53 (14.2)**	**16 (30.2)**	**37 (69.8)**	**<0.0001**
Myocardial Infarction, n(%)	**7 (1.9)**	**4 (57.1)**	**3 (42.9)**	**1.0**
Stroke, n(%)	**18 (4.8)**	**9 (50)**	**9 (50)**	**0.809**
Peripheral vasculopathy	**6 (1.6)**	**2 (33.3)**	**4 (66.7)**	**0.418**
Angina, n(%)	**10 (2.7)**	**3 (30)**	**7 (70)**	**0.196**

Table 15. *Characteristic table for all SLE/RA patients with PD or Healthy controls.*

Patients with concomitant SLE and PD were more prevalent with smoking (58.6%), Diabetes (55%), Hypertension (53.3%), Hypercholesterolemia (68.6%), statin use (68.6%), Myocardial infarction (60%) angina (71.4%) (table 16).

Characteristic table for SLE patients (n=210)			
Variable	**Healthy**	**PD**	**p-value**
Age, years	**36.8±12.3**	**44.4±15.6**	**0.001**
Gender, Female (%)	**74 (55.6)**	**59 (44.4)**	**0.857**
Current Smoking, n(%)	**12 (41.4)**	**17 (58.6)**	**0.112**
Diabetes, n(%)	**9 (45)**	**11 (55)**	**0.353**
Hypertension, n(%)	**28 (46.7)**	**32 (53.3)**	**0.126**
Hypercholesterolemia, n(%)	**15 (36.6)**	**26 (63.4)**	**0.009**
Statin use, n(%)	**11 (31.4)**	**24 (68.6)**	**0.003**
Myocardial Infarction, n(%)	**2 (40)**	**3 (60)**	**0.658**
Stroke, n(%)	**5 (50)**	**5 (50)**	**0.755**
Peripheral vasculopathy	**2 (50)**	**2 (50)**	**1**
Angina, n(%)	**2 (28.6)**	**5 (71.4)**	**0.246**

Table 16. *Characteristic table for SLE patients with PD and Healthy controls*

Patients with RA were associated with multiple factors including smoking (70%), diabetes (65.4%), Hypertension (68.4 %), Hypercholesterolemia (80%), statin use, angina (66.7%) (table 17).

Characteristic Table for RA patients (n=174)			
Variable	**Healthy**	**PD**	**p-value**
Age, years	**50.8±17.3**	**54.8±14.8**	**0.069**
Gender, Female (%)	**99 (55.9)**	**78 (44.1)**	**0.407**
Current Smoking, n(%)	**3 (30)**	**7 (70)**	**0.186**
Diabetes, n(%)	**0 (34.6)**	**4 (65.4)**	**0.040**
Hypertension, n(%)	**12 (31.6)**	**26 (68.4)**	**0.001**
Hypercholesterolemia, n(%)	**3 (20)**	**12 (80)**	**0.005**
Statin use, n(%)	**5 (29.4)**	**12 (70.6)**	**0.037**
Myocardial Infarction, n(%)	**2 (100)**	**0 (0)**	**0.501**
Stroke, n(%)	**3 (50)**	**3 (50)**	**1**
Peripheral vasculopathy	**0 (0)**	**1 (100)**	**0.452**
Angina, n(%)	**1 (33.3)**	**2 (66.7)**	**0.592**

Table 17. *Characteristic table for RA patients divided into healthy controls and PD*.

Analysis of the patients with SLE/RA and concomitant PD when compared to healthy controls (RA with no PD) confirmed that there was no statistically significant difference in the prevalence of PD between the two patients' groups. SLE/RA patients who reported PD had increased CRP serum levels compared to the ones who did not report symptoms of PD (p = 0.03). Similarly, in the RA patient group alone, patients with PD had higher ESR (p = 0.008) than the RA patients with no PD (figures 22 and 23).

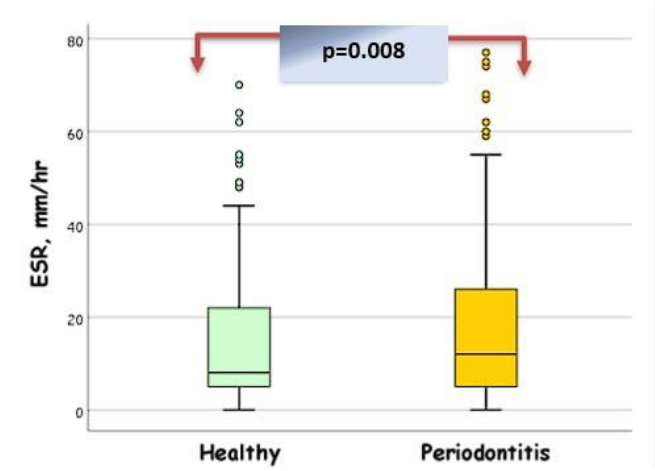


Figure 22. *Box and whisker plots of ESR (mm/hr) in patients with RA (n=174) based on whether they presented with PD or not (Healthy). Median and 25th/75th values are presented together with outliers (open circles) and comparison was performed using t-test statistics. Diagnosis of PD in patients with RA was associated with greater Erythrocytes Sedimentation Rate (ESR) (9.8±10.0 vs 18.3±16.6, p = 0.008).*

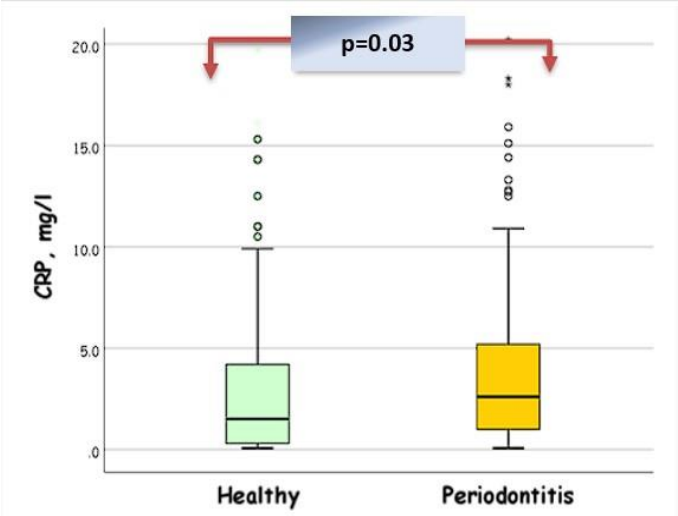


Figure 23. *Box and whisker plots of CRP (mg/l) in patients with RA and SLE (n=171) based on whether they presented with PD or not (Healthy). Median and 25th/75th values are presented together with outliers (open circles) and comparison was performed using t-test statistics. Diagnosis of PD was associated with increased serum levels of C-reactive protein (2.8±3.3 vs 4.0±4.4, **p = 0.03) in the SLE/RA patients.*

Discussion

This survey confirmed that although there is no difference in prevalence of PD in patients with SLE or RA, when periodontitis is diagnosed as co-morbidity there might be a greater systemic inflammatory state. A bi-directional association could be hypothesized between PD and these chronic inflammatory diseases.

A self-reported rating of patients' oral health questionnaire was used to describe the patients' perception of the severity of PD. The self-reported questions were used to identify mild or greater severity of PD. The questionnaire was 85% sensitive and 58% specific and developed an AUROCC (area under the receiver operator characteristic curve) of (0.81) and remaining four questions were 95% sensitive and 30% specific with 0.82 (AUROCC) [284]. The value for AUROCC of the sensitivity and specificity of test indicates the validation and good quality of the questionnaire.

In addition, the self-reported Oral Health questionnaire-based study [284] concluded that there was no statistically significant difference observed in the prevalence of PD between the two patients' groups (SLE & RA). The data findings of the patients with PD and

SLE/RA indicates that the PD was strongly associated with common risk factors including smoking, high cholesterol, statin use, hypertension, and CVD risk factors. It is observed that the amplification of the inflammatory response including higher levels of CRP and ESR have significantly found to be linked with PD in patients with SLE and RA.

Previous evidence indicated that there is a possible link between PD and RA. It has been suggested that patients with RA have a higher risk of developing or presenting with PD [286]. However, only a few studies assessed periodontal status in SLE patients. Ceccarelli et al. stated that there were no indications that patients with SLE are more susceptible to PD than healthy controlled patients [287]. In contrast, other authors found that PD was more prevalent in patients with SLE than in healthy people [288]. Isola et al. suggested that the treatment of PD could impact SLE by reducing both disease activity and SLEDAI [289]. This evidence is in favour of a bi-directional association between these two chronic inflammatory diseases, however further and definitive research should be performed in order to confirm the nature of this association.

Despite the relatively good number of participants included in this survey, one of the limitations of this study was the inclusion of some self-reported measures of periodontal severity which would need further validation in the future. The prevalence of PD instead was evaluated using a validated questionnaire as outlined in the previous chapter.

4.4 Conclusion

PD is known to be an inflammatory disease. SLE and RA are rheumatic diseases that present the similar inflammatory mechanism. Common etiological factors do have a negative impact and can influence the intensity of the diseases. In addition, according to the current evidence PD might contribute to an amplified inflammatory profile in patients with RA and SLE. However, further evidence needs to be gathered on this topic via additional observational and experimental trials.

Chapter 5: Association of Periodontitis with Systemic Lupus erythematosus in a Large U.S. population-based Survey (NHANES III)

Study IV: Association of Periodontitis with Systemic Lupus erythematosus in a Large U.S. population-based Survey (NHANES III)

5. Introduction

Some studies suggested a potential association between PD and SLE. Despite having different aetiologies, the existence of similar inflammatory destructive mechanisms could support an association between PD and SLE [8, 100].

Evidence suggested that both PD and SLE share the similar inflammatory profile [7]. PD (PD) is a chronic inflammatory disease which causes the destruction of the periodontal tissues and alveolar bone which eventually leads to loss of teeth [278].

The incidence and prevalence of PD varies due to case misclassification, bias, number of sites and teeth examined [290] The measurement of loss of the periodontal attachment has been set as a gold standard from the Canadian Health Measures Survey (2007-2009) for reporting the prevalence of PD [291]. However, for the estimation of PD in the United states, National Health and Nutrition Examination Survey (NHANES) considered the clinical attachment loss (CAL) and periodontal probing depth (PPD) at six sites of all teeth except third molars [292]. The Prevalence of PD around the globe is around 20-50% of total population [293]. Other Oral manifestations that are observed in SLE patients might include raised keratotic plaque, honeycomb plaque, oral ulceration, purpura, nonspecific erythema, cheilitis, and petechiae [294].

SLE is a chronic inflammatory illness that affects majorly the joints, skin and internal organs [295]. SLE is known to be a complex auto-immune disease with diverse clinical manifestations and unknown aetiology however, according to the current evidence SLE is found in more genetically susceptible individuals due to the stimulation of an inflammatory response by an environmental stimulus [296] . Symptoms that are common

in SLE patients include rash, fever, vesiculobullous lesions, weight loss, alopecia and glomerulonephritis [297]. In addition, 75% of the SLE patients suffers from arthralgia and arthritis with migratory arthritis [298].

SLE is observed more common in females than males [299, 300] with an onset between 15-40 years of age [298]. In 2010, the prevalence rate of SLE was reported by Sing lau et al. which was 30-50 cases in every 100,000 whereas incidence rates were between 0.9% to 3.1% among every 100,000 per year in Asian populations [301]. However, a prevalence rate of 19.28 per 100,000 have been reported in 2003, Saudi Arabia [302]. Overall a prevalence rate of 15-20 per every 100,000 has been reported in the world [303].

Various studies have reported a common immune response in patients with PD and SLE. Evidence suggested a common immune response has been observed in patients with PD and SLE [19]. At the affected sites, large number of immune cells with high levels of (B cells) have been observed in both the diseases. Furthermore, high number of ANCA (anti-neutrophil cytoplasm antibodies), metalloproteinase modified formation of the of cytokines, increased levels of CRP (C-reactive protein) and TNF-α are found in the circulation [231]. Studies have reported that PD influences the production of the fibrinogen, cytokines, and CRP (C-reactive protein) which are associated with the initiation of atherosclerosis [3].However, there is no significant evidence have reported on the association between PD and SLE. Only one randomized controlled clinical trial by *Fabbri et al.* have reported the effect of the periodontal treatment on SLE. The results from the study showed there was a significant effect of the periodontal treatment on improving SLE disease activity [173]. However, evidence from the intervention trials is limited. The primary aim of this study was to investigate the association between PD and SLE using a representative survey (NHANES 1989-1994) of the US population. The secondary aim was to ascertain the role of systemic inflammation in mediating this association between PD and SLE.

5.1 Materials and Methods

NHANES, a population-based survey (1988-1994) has been analysed to ascertain a possible association between PD and SLE.

5.2 Survey design and study population

Database from the NHANES III (National Health and Nutrition Examination Survey, US (1988-1994) was obtained to analyse the risk factors for SLE. NHANES is a representative sample of the civilian non-institutionalised population who are aged from 2 months or older in the United States. 20,050 participants aged (17 years and older) completed both the medical examinations and household interviews. Only 40 patients self-reported lupus as a diagnosis which indicates a prevalence of 241 per 100,000 cases (*n* = 40; 95% confidence interval: 133–349 per 100,000). The only 5 participants with undefined SLE status have been excluded from the analyses, as well as pregnant women and those individuals reporting cardiac condition or any medical condition requiring antibiotic coverage before the dental examination. From a total of 13,994 participants who had a periodontal assessment, our final study sample was of 13,677 individuals. PD was defined using criteria recommended by the (AAP) American Academy of Periodontology. Characteristic assessment was based on the following components: age, sex, bmi, central obesity (waist circumference >102 cm for males and >88 cm for females); hypertriglyceridemia (triglycerides >150mg/dl); low HDL- cholesterol (<40 mg/dl for men and <50 for women); high blood pressure (systolic: >130 mm Hg or diastolic: >85 mm Hg or on blood pressure medication); and high plasma glucose (>110 g/dl) (7) and CRP levels higher than 3.0 mg/L and antibody biomarkers.

Reproducible periodontal measures were done on dentate participants on randomly assigned half-mouths by calibrated examiners as previously described in the NHANES procedures manuals [304] Among a plethora of case definitions of PD [305, 306], we used a recently reported definition of PD suited for epidemiologic surveys [305] that is: for moderate PD: two sites not on the same tooth with loss of periodontal attachment 4 mm or greater or one site with gingival probing depth 4 mm or greater and for severe PD: two sites not on the same tooth with loss of periodontal attachment 6 mm or greater and at least one site with gingival probing depth 4 mm or greater (28). We also used continuous measures of periodontal health/disease as proxy for systemic exposure of PD [62]. Proportion of periodontal sites with periodontal pockets 4 mm or greater per number of examined sites and proportion of gingival bleeding sites per number of examined sites were included in the analyses.

The STATA 8.0 (StataCorp, College Station, TX) statistical program was used for all analyses (estimated prevalence, logistic regression), considering population weights and adjustment for the complex sampling design. Logistic regression analyses were used to assess the associations between the presence of SLE and its components (dependent variables) with the different measures of periodontal disease adjusted for the effect of other variables (age, sex, BMI, waist, years of education, smoking, ethnicity, general systemic conditions were used as covariates in all multivariate models. General conditions were assessed by aggregate binary variables to note the presence of chronic heart diseases, cancer, diabetes, stroke, emphysema, asthma, arthritis, SLE, thyroid disease, and goitre [307]. Participants were grouped into three groups according to cigarette smoking: current smokers, non-smokers, and no respondents. For each outcome variable (SLE) and each of the individual components), analyses were conducted for the whole available sample, and the different regression models were based on the same number of subjects to ensure comparability.

5.3 Results

5.3.1 Characteristics of study Population (Table 18)

Participants with no or mild PD were predominantly women (47.48%) however, higher percentages for males in moderate and severe PD (4.69% and 1.06%) were observed. Mean age of the participants for Mild or no PD were older than 40 years old whereas older than 54 and 52 years of age for moderate and severe PD respectively, with lower education background and Whites have been presented with the higher prevalence of PD among Black, Mexican and other. 90.5 % of the participants had chronic medical conditions with no or mild PD whereas, 7.8% with moderate PD and 1.56 % with severe PD. Blood pressure, Serum HDL Cholesterol (mg/dl), Serum Triglycerides (mg/dl), C-reactive Protein and blood glucose levels were observed in the normal range for PD however, the participants with PD were presented as borderline overweight according to the BMI values. For SLE, older age was observed to be associated with lupus as compared to the younger age.

5.3.2 Logistic Regression Analysis (Table 18, 19, 20, 21 & 22)

Multiple logistic regression models confirmed an association between moderate PD and age, gender, waist circumference, BMI and Smoking. Never smoking patients was relatively low in lupus patients than non-lupus patients. Out of 40 SLE patients, 25 was diagnosed with PD (62.5%). Moderate PD was associated with SLE with higher odds of 6.8 [95%CI 1.5, 31.4]. Multivariate analysis including ethnicity confirmed a positive but not statistically significant association of moderate PD with SLE (OR=5.8 ,95%CI 1.0, 35.6). PD was more common in Males whereas SLE is known to be more common in females [88]. No association between loss of clinical attachment, periodontal pocket depth and bleeding score were observed in self-reported SLE patients with PD. Whereas other factors do not seem to differ in patients with SLE and PD or Non-PD.

(NHANES 1988-1994)			
Variables	**No or Mild PD (n = 11758)**	**Moderate PD (n = 1582)**	**Severe PD (n = 337)**
Categorical % (N.o)		**CI 95%**	
Gender			
Males	43.22 [42.12, 44.32]	4.69 [4.09, 5.38]	1.06 [8.5, 1.33]
females	47.38 [46.37, 48.38]	3.15 [2.74, 3.63]	0.5 [0.36, 06.8]
Ethnicity			
White	67.72 [65.02, 70.31]	5.38 [4.67, 6.18]	0.96 [0.71, 0.129]
Black	9.38 [8.73, 11.05]	1.30 [1.19, 1.61]	0.35 [0.27, 0.45]
Mexican	0.542 [4.58, 6.39]	0.45 [0.36, 0.57]	6.4e-04 [4.8e-04, 8.6e-04]
Other	7.63 [6.2, 9.35]	0.64 [0.41, 0.98]	0.19 [9.0e-04, 0.04]
Education (years)	90.6 [89.51, 91.58]	7.84 [7.02, 8.73]	1.57 [1.29, 1.91]
Smoking			
Never	65.72 [63.81, 67.59]	4.36 [3.76, 5.05]	7.3 [5.6, 09.5]

Current	24.87 [23.23, 26.59]	3.49 [2.99, 4.07]	0.83 [0.64, 1.07]
Chronic Medical Conditions	90.59 [89.51, 91.57]	7.85 [7.04, 8.74]	1.56 [1.28, 1.9]
Continuous (Mean±SD)			
Age (Years)	40.1±0.4	54.2±0.7	52.3±1.4
BMI (kg/m^2)	26.4±0.1	27.3±0.2	28.1±0.6
SBP (mm/hg)	119.3±0.4	129.6±0.9	130.1±1.4
DBP (mm/hg)	73.9±0.2	76.0±0.4	77.1±0.8
HbA1c (%)	0.1±0.0	0.2±0.0	0.2±0.0
Serum HDL Cholesterol (mg/dl)	51.0±0.4	48.6±0.7	47.3±1.7
Serum Triglycerides (mg/dl)	134.4±2.1	162.6±7.6	162.1±11.5
C-reactive Protein	0.4±0.0	0.5±0.0	0.5±0.0

Table 18. *Baseline Characteristics of Participants according to the diagnosis of PD (n = 13,677)*

Variable	**Univariate risk Factors analysis**		**Multivariate risk factors analysis**	
Age	**Odds Ratio**	**[95% Conf. Interval]**	**Odds Ratio**	**[95% Conf. Interval]**
	1.0	[1.0, 1.0]	1.0	[1.0, 1.1]
Sex (Females)	2.4	[0.7, 8.8]	1.9	[0.5, 6.7]
Ethnicity			0.6	[0.4, 1.0]
Black	0.8	[0.3, 1.8]	-	-

Mexican	0.7	[0.3, 1.9]	-	-
BMI	1.0	[0.9, 1.1]	1.0	[0.9, 1.1]
Waist Circumference	1.0	[1.0, 1.0]	-	-
Smoking	0.7	[0.2, 0.6]	1.2	[0.4, 3.6]
Moderate PD	6.8	[1.5, 31.4]	5.8	[1.0, 35.6]

Table 19. *Logistic regression model of variables for* SLE (n=40) *from NHANES 111 (N = 13,677)*

Variable	Multivariate risk Factors analysis	
	Odds Ratio	**[95% Conf. Interval]**
Sex (Females)	1.6	[0.4, 6.7]
Ethnicity	0.7	[0.5, 1.0]
Smoking	1.6	[0.4, 5.5]
Bleeding on probing (BOP)	0.7	[0.4, 1.0]

Table 20. *Logistic regression model of Multivariate risk factors for* SLE *from NHANES 111 (N = 13,677)*

Variable	Multivariate risk Factors analysis	
Sex (Females)	**Odds Ratio**	**[95% Conf. Interval]**
	1.6	[0.4, 6.7]
Ethnicity	0.7	[0.5, 1.0]
Smoking	1.7	[0.5, 6.1]

Periodontal Pocket depth (PPD)	0.9	[0.5, 1.4]

Table 21: *Logistic regression model of Multivariate risk factors for* SLE *from NHANES 111 (N = 13,677)*

Variable	**Multivariate risk Factors analysis**	
Sex Females	Odds Ratio	[95% Conf. Interval]
	1.8	[0.5, 6.7]
Ethnicity	0.6	[0.4, 1.0]
Smoking	1.3	[0.5, 3.5]
Clinical Attachment Loss (CAL)	1.2	[0.9, 1.45]

Table 22: *Logistic regression model of Multivariate risk factors for* SLE *from NHANES 111 (N = 13,677)*

5.3.3 Linear Regression Analysis

Linear regression analysis confirmed that moderate PD was associated with SLE with ($p < 0.015$). The association between PD and SLE were mediated by age ($p<0.42$) and gender ($p<0.33$).

Linear regression data analysis suggested a gradual increase in the mean percentage of *Pg* antibodies from mild to severe PD. Whereas *Aa (mix, 29523, Y4)* antibodies were much higher in Moderate PD and decreasing at the severe stage of PD. Other antibodies that were found in PD were *P. Intermedia, P. Nigrescens, T. Frosthyia, F. Nucleatum, S. Oralis, M. Micros, S. Rectus, E Corrodens, E. Nodatum, S. Intermedius,*

C. Ochracea, V. Parvula, A, Naeslundii, P. Melaninogencia, S. Noxia, T. Denticola, S. Mutans. (Table 23).

S No.	***Antibodies***	PD Diagnosis	Mean %	Std. Err.	95% Conf. Interval	
1	*P. gingivalis (Pg) mix antibody*	No/Mild	19.08	163.9	1578.6	2237.3
		Moderate	61.21	2603.7	889.2	11353.9
		Severe	84.64	2108.5	4227.6	12701.8
2	*P. intermedia (Pi) antibody*	No/Mild	7.31	73.4	583.6	878.5
		Moderate	7.81	80.8	619.4	944.0
		Severe	11.06	270.5	563.1	1650.2
3	*P. nigrescens (Pn) antibody*	No/Mild	5.24	49.2	425.1	622.8
		Moderate	5.11	61.4	387.8	634.5
		Severe	5.28	125.6	276.5	781.3
4	*T. forsythia (Tf) antibody*	No/Mild	2.65	21.0	223.3	307.8
		Moderate	2.69	34.2	200.4	337.9
		Severe	3.09	67.1	174.6	444.4
5	*A. actinomycetemcomitans (Aa) mix antibody*	No/Mild	43.01	1042.5	2206.7	6396.7
		Moderate	99.09	6024.9	-2198.3	22016.7
		Severe	28.09	835.0	1131.7	4487.7
6	*A. actinomycetemcomitans (Aa) 29523 antibody*	No/Mild	22.13	350.9	1508.8	2919.0
		Moderate	31.75	1273.2	616.5	5733.6
		Severe	11.31	176.1	777.7	1485.4

7	*A. actinomycetemcomitans (Aa) Y4 antibody*	No/Mild	19.16	196.4	1521.7	2310.8
		Moderate	21.16	407.3	1298.1	2935.2
		Severe	13.80	173.3	1032.0	1728.6
S No.	***Antibodies***	**PD Diagnosis**	**Mean %**	**Std. Err.**	**95% Conf. Interval**	
8	*F. nucleatum (Fn) antibody*	No/Mild	2.92	29.0	233.9	350.4
		Moderate	2.63	26.5	210.6	317.0
		Severe	3.19	109.5	99.7	539.7
9	*S. oralis (So) antibody*	No/Mild	1.99	24.2	151.2	248.6
		Moderate	2.07	24.2	158.5	255.7
		Severe	1.63	31.4	100.7	226.9
10	*M. micros (Mm) antibody*	No/Mild	6.90	120.2	448.7	931.6
		Moderate	10.74	256.5	558.9	1589.8
		Severe	5.57.6	134.5	287.4	827.9
11	*C. rectus (Cr) antibody*	No/Mild	2.68	33.4	201.2	335.6
		Moderate	3.17	40.2	236.8	398.5
		Severe	7.57	249.3	256.2	1258.2
12	*E. corrodens (Ec) antibody*	No/Mild	4.18	44.0	330.1	506.9
		Moderate	4.43	53.8	335.1	551.2
		Severe	3.90	61.5	267.1	514.1
13	*E. nodatum (En) antibody*	No/Mild	69.22	446.7	6024.6	7820.0
		Moderate	57.19	927.5	3855.3	7583.1
		Severe	26.82	612.6	1451.5	3913.6
14	*S. intermedius (Si) antibody*	No/Mild	7.07	94.9	516.3	897.7

		Moderate	9.2	219.8	478.9	1362.3
		Severe	5.79	131.7	315.0	844.3
S No.	***Antibodies***	**PD Diagnosis**	**Mean %**	**Std. Err.**	**95% Conf. Interval**	
15	*C. ochracea (Co) antibody*	No/Mild	3.75	50.9	272.8	477.4
		Moderate	4.28	132.7	161.6	694.9
		Severe	3.01	46.0	209.0	393.8
16	*V. parvula (Vp) antibody*	No/Mild	0.93	7.0	78.9	107.1
		Moderate	1.03	12.3	78.9	128.3
		Severe	0.92	12.0	68.8	117.1
17	*A. naeslundii (An) antibody*	No/Mild	25.6	385.4	1789.2	3338.1
		Moderate	23.83	731.5	913.2	3853.4
		Severe	16.92	458.8	771.0	2614.8
18	*P. melaninogenica (Pm) antibody*	No/Mild	5.61.0	63.5	433.4	688.7
		Moderate	8.14.8	272.4	267.4	1362.2
		Severe	5.26.7	90.3	345.2	708.2
19	*S. noxia (Sn) antibody*	No/Mild	8.48	602.7	-362.9	2059.5
		Moderate	2.70.2	85.6	98.2	442.1
		Severe	1.39.8	47.7	44.0	235.7
20	*T. denticola (Td) antibody*	No/Mild	5.44	146.6	249.3	838.6
		Moderate	3.63	47.2	268.1	458.0
		Severe	4.07	81.3	243.9	570.7

21	*Mutans (Sm) antibody*	No/Mild	2.42	39.8	162.1	322.1
		Moderate	2.46	31.8	182.2	310.2
		Severe	1.53	18.4	116.4	190.3

Table 23. *Linear regression model analysis showing mean (%), standard error and confidence interval of the periodontal antibodies dominantly present at mild, moderate, and severe PD.*

5.4 Discussion

The analysis of this large US population survey (NHANES) available with dental and general health data elaborated that both categorical and continuous characteristics of PD including serum levels of PD pathogens were associated with SLE independent of age, gender, BMI, waist circumference and smoking differences. Participants with moderate PD had higher odds for SLE when compared with no or mild and severe PD.

These results agreed with current evidence suggesting an association between PD and SLE [236]. The magnitude of this association seemed to be affected strongly by the inadequacy of the current definitions of PD and continuous measures of the periodontal health.

Systemic inflammation as defined hs-CRP did not seem to mediate the association between PD and SLE. However, Bansal et al. in 2014 observed higher serum CRP levels in patients with PD and SLE [308].

SLE is a challenging condition. The diversity of the clinical features and the complexity of the factors which include genetic, hormonal and environmental that are responsible to cause or worsen the condition along with array of auto-antibodies associated with SLE [309]. PD is a clinical condition that causes inflammation of the periodontal tissues which include the periodontal ligament, gingiva, cementum, and the alveolar bone. The severity of the disease may lead to the loss of teeth [310]. Rhodus and Johnson in their study observed that 93.8% of SLE patients had PD [311]. A Japanese study presented a prevalence of PD of 70% in SLE patients when compared to 30% participants had PD in the general population [100].Higher disease activity of SLE measured by Systemic SLE erythematosus Disease Activity Index (SLEDAI) [312], have shown worse periodontal

disease [231] and non-surgical treatment of PD improves SLEDAI scores at 3 months [173].

A novel finding of this analysis is the association between periodontal pathogens antibody levels and the co-existence of self-reported SLE. Similar results including elevated IgA antibody levels against periodontal pathogens were found to be associated with persistent PD and active tissue destruction [313]. The significance of the serum IgA antibodies elevation against the pathogens is still unknown. A previous study by Pussinen et al. in 2004 suggested that the aggressive form of PD is usually associated with higher levels of *A. actinomycetemcomitans* specifically at young age (before 35 years) and *P. Gingivalis* are found more in the adults and can also be associated with the incidence of the other systemic conditions e.g., Stroke [314]. Our study has confirmed this observation by the linear regression analysis of the antibodies which has shown a gradual increase of antibodies against *P. Gingivalis* from mild to severe periodontitis. In 2018, Bagavant et al. has suggested that the higher levels of IgG antibodies were found against the periodontal pathogen *A. actinomycetemcomitans* and higher disease activity (SLEDAI) in SLE patients [201]. Previous studies [173, 311, 315, 316] have also observed the association between periodontal disease and SLE which has been supported with this study.

Bagavant et al. also suggested that the presence of anti-dsDNA antibody correlated with anti-*A. actinomycetemcomitans antibodies,* [201]. Konig et al. also confirmed a plausible link between PD and RA suggesting high levels of *A. actinomycetemcomitans* as a possible trigger of RA [214].

A. actinomycetemcomitans has a unique ability to induce citrullination of host proteins. Therefore, *A. actinomycetemcomitans* exposure amplifies the pathogenic immune response by releasing modifies self-antigens [201, 214]. *It has been suggested that the reduction in the morbidity of debilitating systemic diseases might be beneficial for the improvement and management of the periodontal health [317]. In addition, one randomized clinical trial by Fabbri et al. in 2014 suggested that periodontal treatment can improve the* SLE *disease activity in the patients [173].*

This study analysis had several limitations which include the cross-sectional nature of the data which do not allow the causal interpretations due to the indication of a strong association by the data without interfering any information as to the direction of a potential causal pathway. It should also be observed that the diagnostic criteria to define PD including SLE and the medications taken by the participants are intentionally conservative. The data set consist of half mouth recordings which might affect the analysis. Many other cofounding factors (e.g., Oral health behaviour, genetics) were not assessed which could be a cause of excluding the possibility of a factitious association between PD and SLE. Nevertheless, this analysis has observed a significant association between PD and SLE with greater odds. Therefore, further interventional studies can observe the treatment of the PD and its impact in the reduction of the SLE disease activity.

5.5 Conclusion

PD and SLE are closely linked but this association is heavily mediated by age and gender differences. Periodontal pathogen including *A. actinomycetemcomitans* and *P. gingivalis* are suggested to have a dominant role in moderate and severe PD. In addition, it has been suggested that *A. actinomycetemcomitans* are strongly associated with SLE by worsening the SLE disease activity. Further interventional studies are required to ascertain the significance of the treatment of PD and its impact to improve the disease activity of SLE.

Chapter 6: Treatment of Periodontal Diseases in Systemic Lupus Erythematosus. A Pilot Randomized Controlled Clinical Trial Protocols and Methodology

Study V: Treatment of Periodontal Disease in Systemic Lupus Erythematosus. A Pilot Randomized Controlled Clinical Trial Protocols and Methodology

6 Introduction

SLE is defined as a multisystem rheumatic autoimmune condition associated with increased mortality as compared to the general population accompanied with early deaths due to the active onset of the disease that led to the aggressive infections and late deaths occurring due to the cardiovascular disease's complications [318-320]. According to the studies, the advancement of the treatment and the understanding of the disease mechanism have increased the survival age of the SLE patient by 10 years. However, close monitoring and symptom treatment has shown a remarkable improvement with the SLE patients and increased the probability of their survival [321, 322]. Furthermore, cardiovascular diseases (CVD) as a complication of SLE is responsible for most of the deaths and known as the main cause of death for SLE patients. According to the previous studies, CVD has observed to occur more in young adults as compared to the general population [323]. Therefore, continuous monitoring and appropriate treatment is crucial for SLE patients, and it is highly recommended [324]. It is reported that the SLE patients have a 5-10 times higher risk probability to have Cardiovascular events in comparison to the general population [323]. Furthermore, coronary artery calcifications have been observed in SLE patients [325, 326]. These pathological events are observed late in the atherosclerosis process [327, 328]. In atherosclerosis, endothelial dysfunction is known to be the initial stage of the event in SLE patients [329]. Studies have indicated that the early-stage detection of the endothelial dysfunction and prevention could improve the survival rate of the patients. Studies have suggested that the early detection of the cardiovascular events through Flow mediated dilatation (FMD) are crucial in SLE patients who are unaware of the disease [330]. In addition, PD in SLE patients have been

observed as a potential contributor in influencing the inflammatory mechanism of SLE and other life-threatening diseases that include Atherosclerosis [331]. In PD, the host response consists of a chronic inflammatory mechanism that is activated against the pathogenesis of the oral pathogens that is associated with higher number of systemic inflammatory biomarkers [332, 333]. Therefore, it has been suggested that the treatment of PD has the potential to reduce the inflammatory response and profile in SLE patients [173].

6.1 Hypothesis

The aim of our study is to determine baseline comparisons between SLE patients and SLE with PD. The other objectives will include whether PT will affect the endothelial function in SLE patients. The secondary aims will be to evaluate changes in oxidative, inflammatory SLE progression markers and skin lesions related to SLE. This study will provide the first insight into whether treating periodontal disease influences SLE disease activity (SLEDAI) and CV risk factor in a (high-risk population) such as patients with SLE and on the disease itself. These findings would be of relevance to the development of strategies of primary cardiovascular prevention in SLE and improvement in SLE disease activity.

6.2 Recruitment and Methods

6.2.1 Study Population

This study was designed to recruit patients suffering from SLE; some with PD and some without PD. The original plan was to seek permission from the potential participant to inform their GP/GDP or other health care professional of their enrolment in the research study. All the data acquired in the trial was going to be used for research purposes however in case of medically significant findings we will inform participant's General Practitioner and General Dental Practitioner to investigate further. The included population needed to have SLE with no PD and cases with PD and SLE, 18 years of age or over, patients with 4 or more criteria for SLE according to the American College of Rheumatology (ACR) 1997 criteria or SLICC 2012 criteria or biopsy proven lupus nephritis

with one additional supportive test on at least two occasions (positive ANA, anti-dsDNA antibodies or anti-Sm antibodies), presence of moderate to severe PD (at least 30 pockets with Probing depth equal or greater than 5mm) and have voluntarily signed the informed consent. The sample size was not determined by a power calculation due to being a pilot study.

Exclusion criteria included:

Female that was Pregnant or breastfeeding, having fewer than 15 teeth, knowingly had HIV or Hepatitis, not capable to give informed consent, presence of concomitant RA, Sjogren syndrome, diabetes mellitus, Smoking, subject on anticoagulants, subjects on chronic antibiotic therapy or who required antibiotic coverage for periodontal procedures and participants who received periodontal treatment within 6 months from the baseline.

6.3 Ethical approval

The study was approved by the JRO (Joint Research office) committee of UCL, REC (Research Ethics Committee)/HRA (health Research Authority. The trial was published at Clinical trial.gov (https://clinicaltrials.gov/ct2/show/NCT04046172).

Following the Covid-19 Pandemic (lockdown and restrictions) plus the logistical challenges brought by the move from an old to a new clinical and academic facility, it was not possible to start the clinical study and collect data on the association between SLE and PD.

6.4 Trial design

The study was designed as a multi-centre, 2-part clinical trial with two baseline observational groups: 200 participants with SLE and a healthy periodontium (controls) and those with PD and SLE (cases); 30 participants will be selected by randomization would be subsequently entered into a controlled trial with 2 treatment groups for evaluation. The trial would assess the effect of periodontal treatment (PT) on the endothelial function in a population affected by SLE. The study population was going to be recruited among those patients with SLE attending the Department of Rheumatology, UCLH Hospital and the patients with PD and SLE referred by their General Dental

Practitioner to the Eastman Dental Hospital new patient clinic for assessment and treatment. All the participants needed to give written informed consent to the study.

Participants meeting the necessary inclusion/exclusion criteria would be accepted for the study in order to recruit 30 patients suffering from PD and SLE. Participants who consented to this study would undergo a baseline visit in which they will have a comprehensive full mouth periodontal probing depths assessment. In addition, full mouth plaque and gingival bleeding scores will also be calculated. A series of parameters would be recorded (including age, gender, ethnicity, and body mass index). Saliva samples (1 ml) and Blood samples (32 ml) will also be collected for analysis of peripheral blood inflammatory and oxidative biomarkers. Blood cell counts, C-reactive protein, complement levels, dsDNA autoantibodies, kidney and liver function tests will also be performed. The vascular function was going to be assessed by means of an ultrasound scan. After randomization to either Test or Control Group, the test group will undergo periodontal treatment in 2 sessions within a week from each other. Radiographic examination Orthopantomogram (OPG) will be taken at the second visit of patient's visit only. Optical coherence tomography will be done on the patients in visit (2,4 and 6). At 2 months both groups will be reassessed, and the same information and samples taken at baseline will be collected. The test group will undergo additional periodontal treatment visit (3a and 3b) of Intensive periodontal treatment/IPT) within 3 weeks from the 2 months visit. After this visit the Control group will receive the same periodontal treatment (Control periodontal treatment/CPT). At 6 months both groups will be seen for the final study assessment. If at any of the study assessment (2 months and 6 months) participants in the control group show signs of progression of PD they will be treated separately and exited from the trial. After 6 months all the participants will have treatment irrespective of groups, if they require treatment, it will be provided.

6.5 Conclusion

The available literature has suggested a bidirectional link between SLE and PD. The management of PD should be considered in the treatment of the SLE patients. This clinical trial will confirm the causal relationship between the two diseases that will guide

the clinicians to elucidate the biochemical and immunological interaction between SLE and PD.

Chapter 7: Final Discussion, Conclusion, Relevance of the study and Future work

7 Final Discussion and Conclusions

This research programme aimed at exploring a potential bi-directional association between PD and rheumatological diseases (SLE and RA).

In Study I, a panel of different clinical parameters were compared to ascertain a potential clinical association between the two diseases. Higher odds of PD were observed in SLE patients as compared to the control group (without SLE). Previous studies have suggested the higher prevalence of PD in SLE patients and supports the evidence observed in this study [7, 264]. Study I also confirmed an important confounding of this association based on gender differences. As SLE is more common in female participants, evidence suggests that this could be due to a hormone's imbalance (progesterone/testosterone) which in turn might influence body inflammation [265, 266]. This hormonal imbalance could also affect the susceptibility to PD but further research is needed.

When analysing the clinical periodontal parameters, inconclusive evidence exists when comparing the severity of PD in patients with SLE when compared to the patients with PD without SLE [85]. Higher odds of increased CAL and PPD in patients with SLE and PD was observed in a controlled study [91, 250]. However, reduced PPD was reported in other studies [91, 248, 250, 334]. Sojod et al. suggested that the risk of PD is significantly higher in SLE patients compared to the patients without SLE and this was confirmed in a subsequent meta-analysis [264].
This conflicting data might be due to variation in the case definitions used and the clinical parameters collected in patients with PD and difference in the scoring system of SLE disease activity, as well as predisposing factors for coexisting conditions, population and their environmental background [85].

An observational study proposed that patients with PD had increased risk of developing SLE at some point in their lives [253]. However, the evidence was time and dose dependent and there were no statistically significant findings on the odds of having SLE in PD patients. The study focused on smoking as a common risk factor for both PD and SLE whereas, the data lacks the information regarding the patients' status of smoking and its effects on both diseases. In addition, Wang et al. suggested that patients with PD had higher risk of developing SLE than the general population [250]. This evidence has been recently confirmed by a mendelian randomization analysis in a large dataset [335]. Plausible mechanism that can potentially associate SLE, and PD pathogenesis include:

- Auto-reactive (B-cells) and the dysregulation of the several types of immune cells such as neutrophils, macrophages, dendritic cells, T-cells and CD+4 cells [336].
- Anti-inflammatory and inflammatory cytokines imbalance that could be SLE-induced and result in destruction of the tissues [252].
- The systemic auto-immunity imbalance can influence the sub-gingival pathogens [85].

In Study II, a similar analysis confirmed that periodontal clinical parameters were worse in patients with RA with higher DAS28 scores with no earlier diagnosis of PD however, the heterogeneity was high. Diagnosis of PD was associated with worse DAS-28 scores (0.74) as previous studies reported [337, 338]. Indeed, patients with RA have increased risk of PD along with higher CAL and loss of teeth as compared to the general population [339, 340]. Large number of studies including observational, cohort and case control demonstrated an association between PD and RA [341-343]. However, limited evidence on the bi-directional direction of a possible association between worse periodontal clinical parameters and higher DAS-28 scores was found. Periodontal clinical parameters were not recorded in a uniform manner and variation in the case definition of PD introduced significant bias in the analyses. Other studies comparing partial dental examinations and identifying association between PD and RA (NHANES I-III) were previously reported [344-348]. A recent study performed in Taiwan demonstrated higher odds of PD in patients with RA with worse PPD and CAL [349]. Some studies have also indicated the PD severity associated with worse DAS-28 scores [337, 338]. In addition, limited

evidence suggests that treatment of periodontitis could improve DAS-28 scores, ESR, CRP and TNF levels in RA patients [350-353].

Further, it was suggested that PD with its local inflammatory and bacterial burden could influence RA cell pathways and regulatory signalling [354]. Biomarkers and mediators of thrombosis and angiogenesis could be responsible for this potential link (PD affecting RA activity) [355]. Other studies reported higher incidence of PD in patients with RA [356]. This finding however was not confirmed by all studies [357], whilst others focused on the potential role of infections in the pathogenesis of RA by influencing the proteins citrullination [358].

Collectively the evidence suggests a consistent association between PD and RA but whether this is a causal association requires further experimental evidence. It will be crucial to assess whether treatment of PD would influence RA either in the onset/progression and evolution.

When we analysed data from Study III using a self-reported questionnaire, we observed no increased prevalence of PD in both patients with SLE and RA whilst higher CRP and ESR levels were present when both diseases were diagnosed. The study confirmed a potential common role of systemic inflammation as potential driver of both PD and rheumatic diseases [224, 225, 343, 359-361]. Other studies have also debated about the common genetic factors for RA and PD. Common genetic factors including HLA-DR (Human leukocyte Antigen) along with shared polymorphisms and epitope, (epigenetic) alterations were reported in both PD and rheumatic diseases [172].

According to previous evidence, the odds of PD in SLE patients varied between 1.6 and 1.9 [362] when compared to the healthy controls. According to these studies the higher odds of PD were observed in adult population of SLE patients (1.7) [8, 90, 173, 250, 344]. Several mechanisms were proposed to explain the plausible mechanism for PD and SLE [363]. In patients with PD and SLE, infections and risk determinants including genetic, environmental, and hormonal factors play a crucial role in explaining a possible association [85].

Lastly in a large observational study (Study IV) we analysed a large US population suggesting a possible association between PD and self-reported SLE. The study further

highlighted a possible linear pattern of this association not only when analysing clinical measures and case definitions of PD but also higher exposure to common periodontal pathogens especially *P. gingivalis* but also *m. Micros*. Similar evidence confirmed an association between oral pathogens in patients with PD and SLE [215]. *T. denticola, S. sanguinis* and *C. ochracea* were found to be higher in number in healthy individuals without PD as compared to patients with SLE. They also suggested that a higher number of *S. noxia, S. oralis*, and *A. gerencseriae* were found in patients with SLE and RA.

Based on the data from Studies III and IV a moderate association between PD and SLE could be confirmed but limited evidence on the causal nature of this association could be found. Therefore, our group designed and put forward a new clinical trial (Study V) to provide better and robust evidence on the association between PD and SLE as well as whether treatment of PD would impact on SLE activity and the vascular risk profiles of these patients. This study was based on the notion that the treatment of periodontitis has the potential to have a positive impact on the SLEDAI scores and might be able to reduce the inflammation overall in patients with PD and SLE [173, 250]. A study by Pasadas et al., suggested that patients with PD and SLE would present with increased cardiovascular risk [364] and greater incidence of vascular events could be hypothesized [365]. Therefore, in the pilot study we focused not only on understanding whether treatment of PD could influence SLE severity but most importantly if the vascular risk profile could be affected and reversed by the improvement in periodontal health.

Study V was conducted to preliminary assess the prevalence of self-reported periodontitis in patients with Rheumatic diseases. In addition, we wished to increase awareness of the plausible link between SLE and PD amongst medical and dental professionals. According to our observations, almost all the SLE patients and most of the clinicians/dental professionals were unaware of this association between the two diseases. Our intention was also to preliminary assess feasibility of recruiting patients with PD and SLE for possible future research projects. The clinical randomized trial was designed to test the oral and systemic benefit of managing PD in patients with SLE. The Covid-19 pandemic as well as the nature of the disease of the potential participants' condition affected the

potential recruitment for this study. A few lessons were learned, firstly it was obvious that patients with SLE were not willing to come into the hospital for multiple visits. Therefore, we were unable to collect any data from the SLE patients who showed interest to participate in the study but were not able to participate with face-to-face visits. This critical aspect could prove useful for future recruitment plans involving patients with Rheumatic diseases. Providing clear and accessible information about the purpose and potential benefits in participating in such a clinical trial would be considered essential to facilitate recruitment and retention. It became clear that using educational materials explaining the safety measures in place to protect participants, tailor communication to address the specific concerns and needs of immunocompromised individuals, provide opportunities for potential participants to ask questions and express their concerns are even more crucial in this high-risk patients' group. Considering flexible scheduling, including remote monitoring options, and transportation assistance will be important for patients with RA and SLE. Lastly another lesson learned is to define and address financial barriers in participating in such clinical trials and it would be advisable for the future that if funding is secured then it should offer transportation costs or compensation for time and participation.

7.1 Relevance of the Study

This study demonstrates a consistent association between PD and rheumatic diseases. According to the available evidence, however this study confirms only a moderate association between PD and Rheumatological diseases (SLE and RA). In a population that is going to age more and suffer from an increased burden of multimorbidity, this study will contribute to raised awareness amongst general medical and dental practitioners regarding the importance of oral health and management of PD in patients with Rheumatic diseases (SLE and RA). National health services are still lacking strong linkages between medical and dental health professionals but in a not-too-distant future this study would hopefully open the door for common services where patients with SLE and RA could receive oral health awareness information and be referred to dental professionals to improve their oral health. Further as PD could be a modifiable factor for the progression of the rheumatic diseases (SLE and RA) there might also be scope to

improve management of these important diseases and reduce their burden at population level.

7.2 Future Work

Future research efforts in this area could be summarised in the following categories:

- Epidemiological research
- Mechanistic studies
- Experimental evidence
- Service delivery – public health

Epidemiological studies with appropriate sample size (large) and design (prospective-cohort and case/controls) should investigate the temporal association between PD and Rheumatic Diseases with the intent of exploring the nature of the association between these chronic inflammatory diseases.
Future research should also focus on addressing the intricate association between Rheumatic Diseases like SLE, RA and PD by combining clinical, molecular, and epidemiological methods to unravel the complex interplay between these conditions.

Mechanistic studies assessing the impact of the inflammatory and microbial burden at the periodontal level should be performed using samples and experimental models to test a bi-directional association. In particular, microbiome analyses as well as proteomic/metabolomic profiling of saliva/serum of patients with PD and SLE are warranted. Further, as preliminary evidence implicates a keystone pathogen linked to PD (Porphyromonas Gingivalis) as a possible trigger of immuno-modulatory changes linked to the pathogenesis of RA, we wish future studies would focus on the potential impact of exposure to periodontitis pathogens and the onset of SLE or other Rheumatic Diseases.

Experimental research efforts for the future should include the conduct and completion of clinical intervention trials involving patients with SLE and PD and assess whether the impact of PD is not only linked to systemic inflammation but also onto vascular risk parameters. Delivering safely treatment of PD in these patients will show whether the

removal of oral inflammation would be associated as expected with an improved overall vascular function.

Lastly interpretation and dissemination of the above evidence should ultimately serve as basis for the creation of Health Services changes. Specific clinical guidelines aimed at physicians and dental practitioners as to unify medical care for patients with Rheumatic Diseases and including Oral Health promotion strategies from the start of the diagnosis of systemic inflammatory disorders. This would only be accomplished if Healthcare would facilitate communication between the medical and dental professionals as well as empower patients with Rheumatic Diseases as to demand appropriate information and clear instructions on how to manage and improve their oral and general health.

8 Appendices

8.1 Appendix 1: Abstracts presented at different conferences

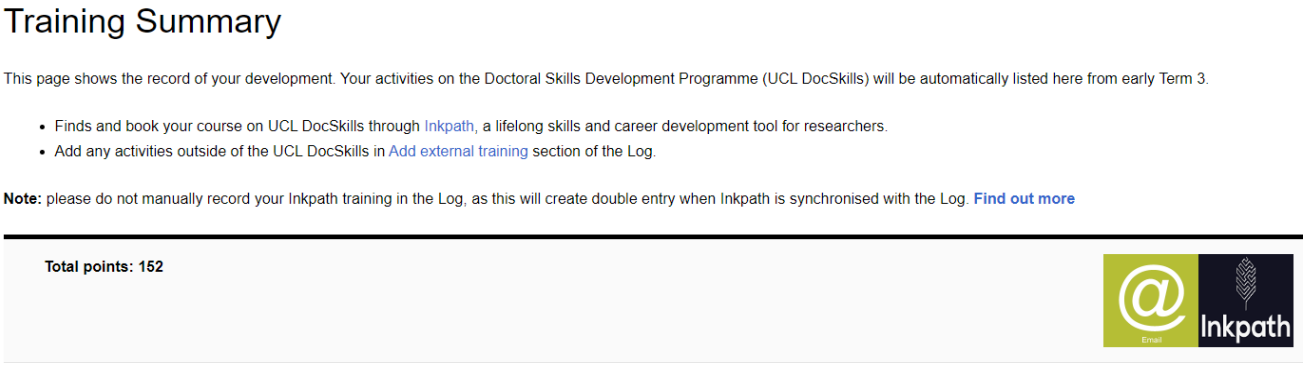
BSODR Abstract 2021 Birmingham

Title: Is Periodontitis a risk factor for Gestational Diabetes Mellitus? A Systematic Review and Meta-Analysis of Cross-sectional and Prospective Studies.

Syed Basit Hussain [1]*, Vanessa Machado [2,3]*, João Botelho [2,3], Syeda Ambreen Zehra [4], José João Mendes [2], Marco Orlandi [1], Francesco D'Aiuto [1]

Background: The nature of the association between periodontitis (PD) and gestational diabetes mellitus (GDM) is still unclear. The aim of this review was to systematically appraise the evidence linking PD and GDM.

Methods: Six databases (PubMed, Google Scholar, Embase, Cinhl, Scopus and CENTRAL) were searched up to February 2021. Observational (cross-sectional and prospective) studies including pregnant women without baseline GDM and diagnosis of periodontitis were included in the review. Risk of bias and publication bias was assessed through the Newcastle-Ottawa Scale. Our primary outcome was the likelihood of PD pregnant women to develop GDM. Secondly, we compared periodontal measures from



GDM and non-GDM patients. Random effects meta-analysis of odds ratio (OR) and of ratio of means (ROM) were performed.

Results: Searches resulted in 12 studies and eight case-control, and four prospective studies met our eligibility criteria. High degree of variability of case definitions of GDM and PD were reported in all studies. Analysis of 2344 pregnant women (including 1601

without GDM and 743 with GDM) confirmed that diagnosis of PD during the first trimester was associated with doubled odds of developing GDM during the last two trimesters (OR=2.41, 95% CI=0.95 to 3.03, p<0.001, I^2=50%). Further GDM was associated with worse clinical periodontal parameters including periodontal probing pocket depth (ROM of 1.10, 95% CI 1.03-1.16), clinical attachment level (ROM 1.15, 95% CI 1.04-1.31), and bleeding on probing (ROM 1.24, 95% CI 1.08-1.42).

Conclusion: PD diagnosis during the first trimester of pregnancy increases the odds of development of GDM. Conversely GDM is associated with poor periodontal health status during pregnancy.

IADR (Virtual)-Abstract 2021

Title: Association of Periodontitis with Systemic Lupus erythematosus in a Large U.S. population-based Survey (NHANES III)

Authors: Syed Basit Hussain[1], Marco Orlandi[1], Coziana Ciurtin[2], Francesco D'Aiuto[1]

1: Periodontology Unit, UCL Eastman Dental Institute, London, UK

2: Rheumatology Unit, UCLH, London, UK

Objective: The primary objective was to assess the association between Systemic Lupus Erythematosus (SLE) and Periodontitis (PD) in a large US survey.

Methods: A representative sample of the US population (n= 13,677, NHANES III, 1988-1994) was retrieved and analysed. The association between PD (exposure) and self-reported SLE (outcome) was assessed using multivariate linear and logistic regression models. Additional exposure included serum antibody levels of common periodontal pathogens.

Results: Participants with periodontitis were more likely to have self-reported SLE (OR 2.6 [95%CI 1.1, 6.0]) compared to those without PD. The association was stronger in those participants with moderate periodontitis diagnosis (OR 6.3 [95%CI 1.4, 28.7]). Logistic regression confirmed that participants with greater clinical attachment levels exhibited greater odds of self-reported lupus (OR 1.04 [95%CI 1.01, 1.08]). Case definition and continuous measures of periodontitis were linked to serum antibody levels of *Pg* and *Aa*. Participants with higher serum antibody levels of *Mm* (OR 1.6 [95%CI 1.0,

2.4]) and lower antibody levels of *Pg* (OR 0.8 [95%CI 0.6, 1.0]) reported higher prevalence of SLE. All findings were independent of age, gender, ethnicity, education, adiposity and smoking differences.

Conclusion: Our data support and association between PD and SLE. Further investigation is required to ascertain the nature of this link and the potential benefit of periodontal treatment on SLE progression and severity.

Keywords: Periodontal Disease, Systemic Disease, Lupus.

Abstract IADR (Virtual) 2020

PERIODONTAL STATUS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A SYSTEMATIC REVIEW WITH META-ANALYSIS

AUTHORS: Syed Basit Hussain[1], João Botelho[3], Vanessa Machado[3], Yago Leira[1], Marco Orlandi[1], Jacopo Buti[1], Coziana Ciurtin[2], Francesco D'Aiuto[1]

Objective: The Primary aim of this systematic review and meta-analysis was to evaluate the severity of periodontitis (PD) by assessing the mean difference of PPD and CAL in patients with Systemic Lupus Erythematosus (SLE) and Controls. Secondary aim was to evaluate the risk ratio towards PD in SLE patients.

Methods: Electronic databases were searched including hand searching of bibliographic references of included papers, related reviews, and journals. Random effects meta-analyses were performed to investigate the association between PD and SLE as assessed by probing pocket depth (PPD), clinical attachment loss (CAL).

Results: 1177 citations and 22 full text articles were screened. 13 articles were included in the qualitative and 9 in the quantitative analysis. Meta-analysis showed that the weighted mean difference (WMD) of PPD (MD=-0.07 mm, 95%CI: -0.44, 0.30, P>0.05) and CAL (MD=-0.10 mm, 95%CI: -0.42, 0.23, P>0.05) between patients diagnosed with SLE and controls was not significant. SLE does not worsens PPD and CAL in PD patients whereas, SLE activity can be accelerated by the presence of PD in patients. Risk ratio has been conducted to assess gender influence towards PD in SLE patients. Meta-regression showed that female SLE patients have higher risk of developing PD (Estimate = 1.83, P <0.001).

Conclusions: Results have showed that patients with SLE have similar periodontal conditions as compared to the healthy controls. It can be suggested that SLE has no influence on the clinical characteristics of periodontal patients. However, female SLE patients have known to have significant higher risk of developing PD.

ABSTRACT IADR (London) 2018

Title: Does Systemic Lupus Erythematosus affect the severity of periodontitis? A systematic review and meta-analysis

Authors: Syed Basit Hussain, Yago Liera, Marco Orlandi, Jacopo Buti, Coziana Ciurtin, Francesco D'Aiuto.

Objective: A literature systematic review and meta-analysis was performed to evaluate the evidence on the severity of periodontitis (PD) in patients affected by Systemic Lupus erythematosus (SLE).

Methods: Electronic database searching, hand searching of bibliographic references of included papers, related reviews, and journals in relation to oral and rheumatology field was carried out. Random effects meta-analyses were performed to investigate the association of SLE, and PD as assessed by probing pocket depth (PPD) and clinical attachment loss (CAL)

Results: 1176 citations and 21 full text articles were screened. 8 were appropriate to be included in the qualitative and 4 in the quantitative analysis as shown in Figure 1. Meta-analysis demonstrated that the diagnosis of SLE was associated with a mean decrease in PPD of 0.78 mm (95% C.I. -1.84, 0.28) and in CAL of 0.99 mm compared to controls (95% C.I. -2.07, 0.09), Figure 2 and 3.

Conclusions: This study reports that SLE does not affect the severity of PD and suggests a non-significant lower probing depth and CAL in SLE compared to healthy controls. This might be related to the anti-inflammatory and immune-modulatory regimen in the SLE population

Abstract For 3m Presentation UCL Doctoral School 2018

Treatment of Periodontal disease in Systemic Lupus Erythematosus

Presenter: Syed Basit Hussain

Background

Systemic Lupus Erythematous (SLE) is an auto-immune disorder. This disease activates the immune cells to damage the healthy tissue of the body. The presentation of the disease can be fever, pain in chest, painful and swollen joints, rash on the face also known as butterfly rash, lymph nodes swelling, stomatitis, hair loss and lethargy. The cause of SLE is unclear till date. Periodontitis is a chronic inflammatory disease with multifactorial aetiology. It is established by presence of gingival inflammation, recession and bone resorption.

Aim

To assess the effect of Periodontal Treatment on population of patients suffering from Systemic Lupus Erythematosus and Periodontitis.

Method

This will be a pilot randomized controlled clinical trial.

Results

Positive or negative association between two inflammatory diseases to be determined as a result of this Pilot RCT. To establish if the treatment of inflammatory periodontal disease results in improvement of SLE condition.

Conclusion:

Periodontal therapy has a potential to improve SLE disease activity.

8.2 Appendix 2: Self-Reported Oral Health Questionnaire

Question	Self-report Oral Health Questions	Abbreviation (Variable Name)	Response Given
1.	Do you think you might have gum disease?	Have gum disease	Yes/No/ Refused/Don't Know
2.	Overall, how would you rate the health of your teeth and gums?	Teeth/gum health	Poor, Refused, Don't Know
3.	Have you ever had treatment for gum disease such as scaling and root planning, sometimes called "deep cleaning"?	Had gum treatment	Yes/No/ Refused/Don't Know
4.	Have you ever had any teeth become loose on their own, without an injury?	Loose tooth	Yes/No/ Refused/Don't Know
5.	Have you ever been told by a dental professional that you lost bone around your teeth?	Lost bone	Yes/No/ Refused/Don't Know
6.	During the past three months, have you noticed a tooth that doesn't look right?	Tooth does not look right	Yes/No/ Refused/Don't Know
7.	Aside from brushing your teeth with a toothbrush, in the last seven days, how many times did you use dental floss or any other device to clean between your teeth?	Floss use	__: Number of days, 7/7 = Refused
8.	Aside from brushing your teeth with a toothbrush, in the last seven days, how many times did you use mouthwash or other dental rinse product that you use to treat dental disease or dental problems?	Mouthwash	Number of days, Refused

8.3 Appendix 3: JRO Protocols for the Randomized Control Trial

Full title of trial	Treatment of Periodontal Disease in Systemic Lupus Erythematosus. A Pilot Randomized Controlled Clinical Trial. (Student Study)
Short title of trial	Treatment of Periodontal Disease in Systemic Lupus Erythematosus.
Version and date of protocol	Version 2.0 (18/02/2021)
Sponsor:	University College London, UCL
Sponsor Reference number	EDGE 123436
Study Registration number	Z6364106/2019/06/124
Funder (s):	Versus Arthritis
Clinicaltrials.gov no:	NCT04046172
Intervention:	Periodontal Treatment
Single site/multi-site:	Multi-centre

Chief investigator:
Name: Dr. Coziana Ciurtin
Consultant Rheumatologist
Email: c.ciurtin@ucl.ac.uk
Address: Unit of Rheumatology,
UCL, Gower Street, London, WC1E6BT
United Kingdom.

Sponsor Representative:
Name: Delasi Apraku
Email: UCLH.randd@nhs.net
Joint Research Office, UCL, 1st Floor Maple House, 149 Tottenham Court Road
London W1T 7NF
Postal address:
Joint Research Office, UCL
Gower Street,
London WC1E 6BT

PROTOCOL VERSION HISTORY

Version Number	Date	Protocol Update Finalised By (insert name of person):	Reasons for Update
1.0	19/06/2019	Syed Basit Hussain	First Draft
2.0	15/03/2021	Marco Orlandi	Second Draft

Substantial changes have been made that are as follows:

1) The study is divided in to 2 parts:

Part 1: Baseline comparisons.

Part 2: Pilot randomized controlled trial.

2) Address of EDH and Sample Storage has been changed.

3) Version and dates have been amended.

4) Trial coordinator has been changed.

5) Software Analysis part has been removed.

6) Flow diagram of the treatment plan has been amended.

7) Total number of participants has been changed from 30 to 200.

8) Amendments in the Appendix 1- Schedule of assessments

9) Added Appendix 2 Self-reported questionnaire

Signatures

The Chief Investigator and the JRO have discussed this protocol. The investigator agrees to perform the investigations and to abide by this protocol.

The investigator agrees to conduct the trial in compliance with the approved protocol, the UK Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the current Research Governance Framework, the Sponsor's SOPs, and other regulatory requirements as amended.

Chief investigator

Dr Coziana Ciurtin

UCL Signature Date 18/02/2021

Sponsor

Sponsor representative

UCL

Signature Date 17/05/2021

Clinical Study Protocol

Trial personnel
See protocol page for Chief Investigator and Sponsor contact details.

Co-Investigator: Prof. Francesco D'Aiuto DMD, PhD
UCL Eastman Dental Institute
21 University Street
London, WC1E 6DE, UK
e-mail: f.daiuto@ucl.ac.uk

Co-Investigator: Dr. Marco Orlandi DMD, PhD
UCL Eastman Dental Institute
21 University Street
London, WC1E 6DE, UK
e-mail: m.orlandi@ucl.ac.uk

Co-Investigator: Dr. Syed Basit Hussain
UCL Eastman Dental Institute
21 University Street
London, WC1E 6DE, UK
e-mail: syed.hussain.16@ucl.ac.uk

Statistician Dr. Jacopo Buti
UCL Eastman Dental Institute
21 University Street
London, WC1E 6DE, UK
e-mail: j.buti@ucl.ac.uk
tel: 02034561103

Lab Details: UCLH
Rheumatology department lab
4th floor
Rayne's Building
5 University street,
London, WC 1E 6JF

Lead Medical and Physics expert: Prof Peter Marsden
Head of Medical Physics & MPE
University College London Hospitals NHS Trust
Medical Physics, EGA Wing, 235 Euston Road, London
NW1 2BU
Tel: 020 3447 2181
Email: peter.marsden@nhs.net

Lead Clinical Radiation expert: Dr. Steve Bandula
Consultant Radiologist
University College London Hospital
235 Euston Road London
NW1 2BU
Tel: 02023 4470242
Email: s.bandula@ucl.ac.uk

Summary

Objectives

The primary objective of this study is to:

Part 1 – Baseline Comparisons

The primary objective of Part 1 is to:

Investigate the association between SLE and a measure of vascular health (brachial artery flow-mediated dilatation) in patients with Systemic Lupus Erythematosus (SLE) and a healthy periodontium compared to patients with SLE and Periodontitis (PD)

Part 2 – Pilot Randomized Clinical Trial

To assess the effect of Periodontal Treatment (PT) on the endothelial function in a population of patients suffering from SLE and PD.

Secondary objectives include:

Part 1 – Baseline Comparisons

The secondary objectives of Part 1 are to investigate the association of:

- Inflammatory biomarkers in saliva and blood and periodontal status in patients with SLE and PD and those with SLE and a healthy periodontium
- The oral microbiome using plaque analysis and periodontal status in patients with SLE and PD and those with SLE and a healthy periodontium.

Part 2 – Pilot Randomized Controlled Trial.

Evaluate the effect of PT on biomarkers of SLE disease severity/progression in a population of patients suffering from SLE and PD.

Evaluate the effect of PT on the B cell panel in a population of patients suffering from SLE and PD.

Evaluate effect of PT on the systemic inflammatory and oxidative profile of a population of patients suffering from SLE and PD.

Evaluate effect of PT on skin lesions in a population of patients suffering from SLE and PD.

Type of Trial

A two-stage efficient clinical trial design: multi-centre, observational, case/control (two groups) and subsequently randomised controlled clinical trial (RCT) with 2 parallel treatment groups.

Trial Design and Methods

Part 1 of the study investigates the association between SLE and a measure of vascular health (brachial artery flow-mediated dilatation) in patients with Systemic Lupus Erythematosus (SLE) and a healthy periodontium compared to patients with SLE and Periodontitis (PD)through FMD and periodontal examination.

Part 2 of the study is pilot randomized control trial which include the following:

Two baseline groups:

- SLE with healthy Periodontium and SLE and PD

Two treatment groups:

- Treatment Groups A: Non-surgical hand debridement
- Treatment Groups B: Scaling and polishing

Patients recruited into the treatment group arm are to attend a screening, baseline, periodontal treatment visit, 2 months follow-up, periodontal treatment visit, 6 months follow up visits.

Clinical assessments, sample collection (blood, saliva, plaque) and special investigations (vascular, microbiological and immunological) will be performed at similar timepoints for all groups

Trial Duration per participant

Total trial duration per participant of approximately 8 months from informed consent to completion, which includes a 6 month follow up period.

Estimated Total Trial Duration

12 Years and 4 months

Planned trial sites

Multi-site.

Total number of participants planned

200 for Part 1 and 30 for Part 2

Main inclusion criteria

- Cases with SLE and PD
- 18 years of age or over
- Patients with 4 or more criteria for JSLE or SLE according to the American College of Rheumatology (ACR) 1997 criteria or SLICC 2012 criteria or biopsy proven lupus nephritis with one additional supportive test on at least two occasions (positive ANA, anti-dsDNA antibodies or anti-Sm antibodies).
- Presence of moderate to severe periodontitis (at least 30 pockets with Probing depth equal or greater than 5mm).
- Subject must have voluntarily signed the informed consent.

Exclusion criteria

- Cases with SLE only
- 18 years of age
- Patients with 4 or more criteria for JSLE or SLE according to the American College of Rheumatology (ACR) 1997 criteria or SLICC 2012 criteria or biopsy proven lupus nephritis with one additional supportive test on at least two occasions (positive ANA, anti-dsDNA antibodies or anti-Sm antibodies).
- Subject must have voluntarily signed the informed consent.
- Pregnancy or breastfeeding.
- Having fewer than 15 teeth (for cases with SLE and PD).
- Subject knowingly has HIV or Hepatitis.
- Subject is not capable to give informed consent.
- Presence of concomitant RA, Sjogren syndrome, diabetes mellitus.
- Smoking.
- Subject on anticoagulants.
- Subjects on chronic antibiotic therapy or who require antibiotic coverage for periodontal procedures.
- Subjects who received periodontal treatment within 6 months from the baseline

Statistical methodology and analysis:

The primary endpoints of this study are in two parts. One part is to quantify the vascular health in response to PD as a static point prior to active therapy compared to participants with SLE and healthy periodontium. The second part is to quantify and compare the clinical and systemic outcomes of cases with SLE and PD following periodontal treatment.

Part 1

Comparative analyses between cases (SLE with PD) and controls (SLE without PD) will be performed using ANOVA analysis. Primary outcome will be the difference in flow –mediated dilatation between groups. Multivariate analysis will be performed to adjust for a number of covariates including age, gender, body mass index, ethnicity and supragingival plaque levels.

All secondary endpoints will be analysed with ANOVA. Pre-specified analyses of secondary outcomes will include descriptive analyses and differences.

Part 2

Primary clinical periodontal outcome will be the difference in mean flow mediated (peri-implant) at 6 months between study groups and analysed by analysis of co-variance model. Age, gender, body mass index, smoking status, ethnicity and dental plaque levels will be included as additional covariates. Pair-wise comparison and between groups differences will be calculated using Tukey HSD corrections. If the normality assumption does not seem reasonable even after transformation of original values, equivalent nonparametric methods will be used.

Primary systemic inflammatory outcome will be Changes in FMD (primary outcome), circulating inflammatory, vascular and oxidative biomarkers (secondary outcomes) will be analysed with analysis of variance for repeated measures using a conservative F-test (Greenhouse-Geisser correction). If a treatment by time interaction will be found, pair-wise comparisons will be performed (Bonferroni–Holm adjustment). Side effects and safety data will be summarized using standard descriptive statistics. Significance will be set to be at $p < 0.05$.

INTRODUCTION AND STUDY RATIONALE

Scientific Background

Systemic lupus erythematosus (SLE) is a multisystem rheumatic autoimmune disease associated with increased mortality as compared with the general population[1,2] with early deaths due to active disease and infections and later deaths mostly related to cardiovascular

(CV) complications[3]. Juvenile systemic lupus erythematosus (JSLE) is characterised by onset before the age of 18, and accounts for 15–20% of all cases of lupus. JSLE has a more aggressive disease presentation than adult-onset SLE and is associated with increased morbidity and mortality [366] Over recent decades, due to advances in treatment and better understanding of disease mechanisms, 10-year survival of patients with SLE has dramatically improved[4,5]. Deaths related to active disease have been progressively decreasing. However, CV morbidity and mortality have shown no such decline and, nowadays, CV disease persists as the main cause of death in patients with SLE. In particular, CV disease develops earlier and in younger people with SLE as compared with the general population[6]. It has been proposed, therefore, that SLE should be considered as a 'CV disease equivalent' such as diabetes mellitus, with more aggressive monitoring and treatment[7].

Overall, patients with SLE have 5–10 times the risk of CV events compared with the background population[7-10]. Manzi et al. found that women aged 35–44 yr. with SLE had a 50-times greater risk of myocardial infarction (MI) relative to healthy women in the Framingham Offspring Study cohort[10].

Although surrogates of atherosclerosis such as coronary artery calcifications and thickened carotid intima are detectable in SLE patients by imaging[11,12], these pathological vascular alterations are considered to occur relatively late in the atherosclerotic process[13,14]. Endothelial dysfunction is the initial stage in the pathogenesis of atherosclerosis, it has been shown to predict future cardiovascular events even when coronary angiograms are radiologically normal[15]. In contrast to established atherosclerosis, endothelial dysfunction can be reversed if traditional cardiovascular risk factors are treated[16].

There is strong evidence that the endothelial dysfunction in vivo occurs in the peripheral and coronary arteries, affecting the resistance and conduit vessels at different atherosclerotic stages[17]. This evidence suggests that endothelial dysfunction in atherosclerosis is a systemic process and is not restricted to the vessels that show clinical manifestations of atherosclerosis[18]. In 1992, Celermajer et al. described a non-invasive method using high-resolution ultrasound image of the brachial artery to study endothelial function. The dilation of brachial artery in response to increased flow (FMD) is dependent on intact endothelial function, whereas glycerol trinitrate (GTN) is a direct smooth-muscle dilator that acts independently of the status of the endothelium[19].

The detection and intervention during the very early stage of atherogenesis may potentially prevent clinical vascular events and improve the survival of patients with SLE. Observational studies investigating FMD suggested that endothelial dysfunction is present in SLE patients

who are naive for vascular events[20]. A recent meta-analysis of 25 case-control studies involving 1,313 SLE patients and 1,012 healthy controls (HC) reported lower FMD in SLE patients compared to HC (SMD −1.077, $p < 0.001$)[21].

Apart from traditional risk factor such as cholesterol profile, hypertension, smoking and diabetes, clinical and experimental data support an additional critical role for inflammation in atherothrombosis[22]. Downstream biomarkers of inflammation such as high sensitivity C-reactive protein and interleukin-6 are associated with an increased risk of cardiovascular events, independent of the cholesterol level.

Periodontitis (PD) is a chronic inflammatory disease initiated by the accumulation of a bacterial biofilm on the tooth surface and perpetuated by a deregulated immuno-inflammatory response. It is currently considered to be one of the most important global oral health burdens, with a reported prevalence ranging from 20% to 50%, in the general population[23,24]. The chronic inflammatory response to the oral bacteria is associated with higher concentrations of systemic inflammatory markers[25,26] and its treatment is related to a lowering in their profile. Therefore, PD has been indicated as a potential contributor in the pathogenesis of chronic and life-threatening diseases, such as atherosclerosis[27]. PD has been associated to a reduced FMD and increased rate of subclinical atherosclerosis[28] and an excess of oxidants coupled with a decreased antioxidant capacity have been documented in the peripheral blood of patients affected by PD[29]. Our group has extensively explored the relationship between PD and vascular health in the past 15 years collaborating with other UCL department such as Vascular Physiology adopting a technique of vascular health assessment (FMD) invented and validated by this research group. In a randomized controlled trial, we have demonstrated a benefit of periodontal therapy on FMD in otherwise healthy patients affected by moderate to severe PD[30] (Figure 1).

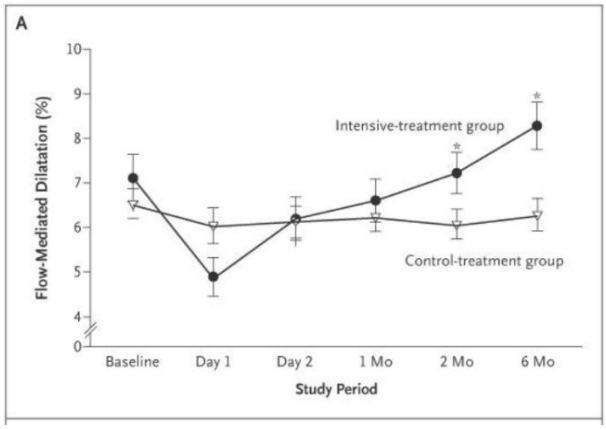


Figure 1 FMD changes after periodontal treatment (adapted from Tonetti et al. N Engl J Med 2007.)

Recent evidence suggests a potential association between PD and systemic rheumatic diseases, mainly rheumatoid arthritis[31]. With regards to SLE, the reported frequency of periodontal disease is ranging from 60 to 93.8%[32]. A recent systematic review and meta-analysis suggested that risk of periodontitis in SLE cases compared

to controls was significantly greater with a risk ratio of 1.76 (95% CI 1.29–2.41, p = 0.0004)[33]. Furthermore, periodontitis could have an adverse effect by enhancing systemic inflammation and increasing the risk of myocardial infarction[34] as well as worsen SLE severity.

The aim of our study is to determine whether PT will ameliorate endothelial function in patients affected by SLE. The secondary aims will be to evaluate changes in oxidative, inflammatory, SLE progression markers and skin lesions related to SLE. This study will provide the first insight into whether treating periodontal disease has an effect on a CV risk factor in a high- risk population such as patients with SLE and on the disease itself. These findings would be of relevance to the development of strategies of primary cardiovascular prevention in SLE[35].

Assessment and management of risk

The table below summarise the risks and mitigations of all tests above standard care that are being performed:

Intervention	Potential risk	Risk Management
• Plaque samples • Saliva samples	I. No significant potential risks have been observed	II. Performed by trained Periodontist. Follow trust standard operational procedures
• Venipunctures	II. Bruising III. Pain IV. Sensitivity	III. Performed by trained Phlebotomist
• Periodontal Treatment	V. Bleeding VI. Gingival recession.	IV. Performed by trained Periodontist. Follow trust standard operational procedures
• Orthopantomogram (OPG)	5 Radiation exposure	V. Performed by trained Medical and Physical expert
• Ultrasound scan of carotids and Brachial arteries (FMD)	6 This rarely might cause headache for a short period of time	VI. Study vascular technician in the Eastman clinical investigation centre
• Optical coherence tomography (OCT)	7 No significant potential risks have been observed	VII. Study clinician and PhD student.

OBJECTIVES

Primary Objective

Part 1 – Baseline Comparisons

The primary objective of Part 1 is to:

• Investigate the association between periodontal status and a measure vascular health (flow-mediated dilatation) in patients with SLE and PD compared to SLE patients with a healthy periodontium.

Part 2 – Treatment Comparisons

The primary objectives (local and systemic) of Part 2 are to:

• Investigate the effect of periodontal treatment (PT) on the endothelial function in a population affected by both juvenile and adult onset of Systemic Lupus Erythematosus (SLE) and PD.

Secondary Objectives

Part 1 – Baseline Comparisons

The secondary objectives of Part 1 are to investigate the association of:

1. Inflammatory biomarkers in saliva and periodontal status in patients with SLE and PD and those with PD and a healthy periodontium
2. The oral microbiome using plaque analysis and periodontal status in patients with SLE and PD and those with PD and a healthy periodontium
3. Part 2 - RCT
4. Evaluate the effect of PT on biomarkers of SLE disease severity/progression (Anti-dsDNA, Complement, IFNα, BLyS and APRIL, Blood cell counts, kidney and liver function) in a population of patients suffering from SLE and PD
5. Evaluate the effect of PT on the B cell subpopulations and function, assessed via flow cytometry in a population of patients suffering from SLE and PD.
6. Evaluate effect of PT on the systemic inflammatory (hs-CRP, IFN-γ, IL-10, IL12-p40, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, BAFF, APRIL, TGFβ1), lipidomic, transcriptomic and oxidative profile (d-ROMs), as well as and markers of endothelial activation (E-Selectin, ICAM-3, P-Selectin, Thrombomodulin) and saliva samples for microbial analysis in a population of patients suffering from SLE and PD.
7. Evaluate effect of PT on skin lesions assessed by Optical Coherence Tomography (OCT) in a population of patients suffering from SLE associated with active skin disease and PD

TRIAL DESIGN

Overall design and plan of the study

This is a multi-centre, 2-part clinical trial with two baseline observational groups: participants with SLE and a healthy periodontium (controls) and those with SLE and PD (cases); the latter are subsequently entered into a randomized controlled trial with 2 treatment groups for evaluation. to assess the effect of periodontal treatment (PT) on the endothelial function in a population affected by Systemic Lupus Erythematosus (SLE). The study population will be recruited screening the outpatients diagnosed with SLE attending the Department of Rheumatology, UCLH Hospital and the patients with SLE and PD will be referred by their General Dental Practitioner to the Eastman Dental Hospital new patient clinic for assessment and treatment. All the participants will need to give written informed consent to the study.

Experimental Design

Participants will be approached by the members of healthcare research team explaining the possibilities to be included in the project. There will be limit in time (24 Hours) to decide whether participate, subject to the recruitment completion. Enough participants meeting the necessary inclusion/exclusion criteria will be accepted for the study in order to recruit 30 patients suffering from SLE and PD. Participants who consent to this study will undergo a baseline visit in which they will have a comprehensive full mouth periodontal probing depths assessment. In addition, full mouth plaque and gingival bleeding scores will also be calculated. A series of parameters will be recorded (including age, gender, ethnicity, and body mass index). Saliva samples (1 ml) and Blood samples (32 ml) will also be collected for analysis of peripheral blood inflammatory and oxidative biomarkers. Blood cell counts, C-reactive protein, complement levels, dsDNA autoantibodies, kidney and liver function tests will also be performed. The vascular function will be assessed by means of an ultrasound scan. After randomization to either Test or Control Group, the test group will undergo periodontal treatment in 2 sessions within a week from each other. Radiographic examination Orthopantomogram (OPG) will be taken at the second visit of patient's visit only. Optical coherence tomography will be done on the patients in visit (2,4 and 6) At 2 months both groups will be reassessed, and the same information and samples taken at baseline will be collected. The test group will undergo additional periodontal treatment visit (3a and 3b) of Intensive periodontal treatment/IPT) within 3 weeks from the 2 months visit. After this visit the Control group will receive the same periodontal treatment (Control periodontal treatment/CPT). At 6 months both groups will be seen for the final study assessment. If at any of the study assessment (2 months and 6 months) participants in the control group show signs of progression

of PD they will be treated separately and exited from the trial. After 6 months all the participants will have treatment irrespective of groups, if they require treatment it will provided.

Selection of Participants

Selection of Study Subjects

This study will include patients suffering from SLE; some with PD and some without PD. We will seek permission from the potential participant to inform their GP/GDP or other health care professional of their enrolment in the research study. All the data acquired in the trial will be used for research purposes however in case of medically significant findings we will inform participant's General Practitioner and General Dental Practitioner to investigate further.

Recruitment

Patients with SLE will be recruited from Rheumatology outpatient clinics at UCLH. Participants will be approached by research staff on the delegation log.

Informed Consent

It is the responsibility of the Investigator, or a person delegated by the Investigator to obtain written informed consent from each participant prior to participation in the trial, following adequate explanation of the aims, methods, anticipated benefits and potential risks of the trial.

The person taking consent will be suitably qualified and experienced and will have been delegated this duty by the CI/ PI on the Staff Delegation log.

a patient refuses to consent any procedure during the study, that patient will be drawn from the study.

There will be no limit of time for the participant to decide whether to participate in the study. However, the minimum of amount of time will be 24 hours.

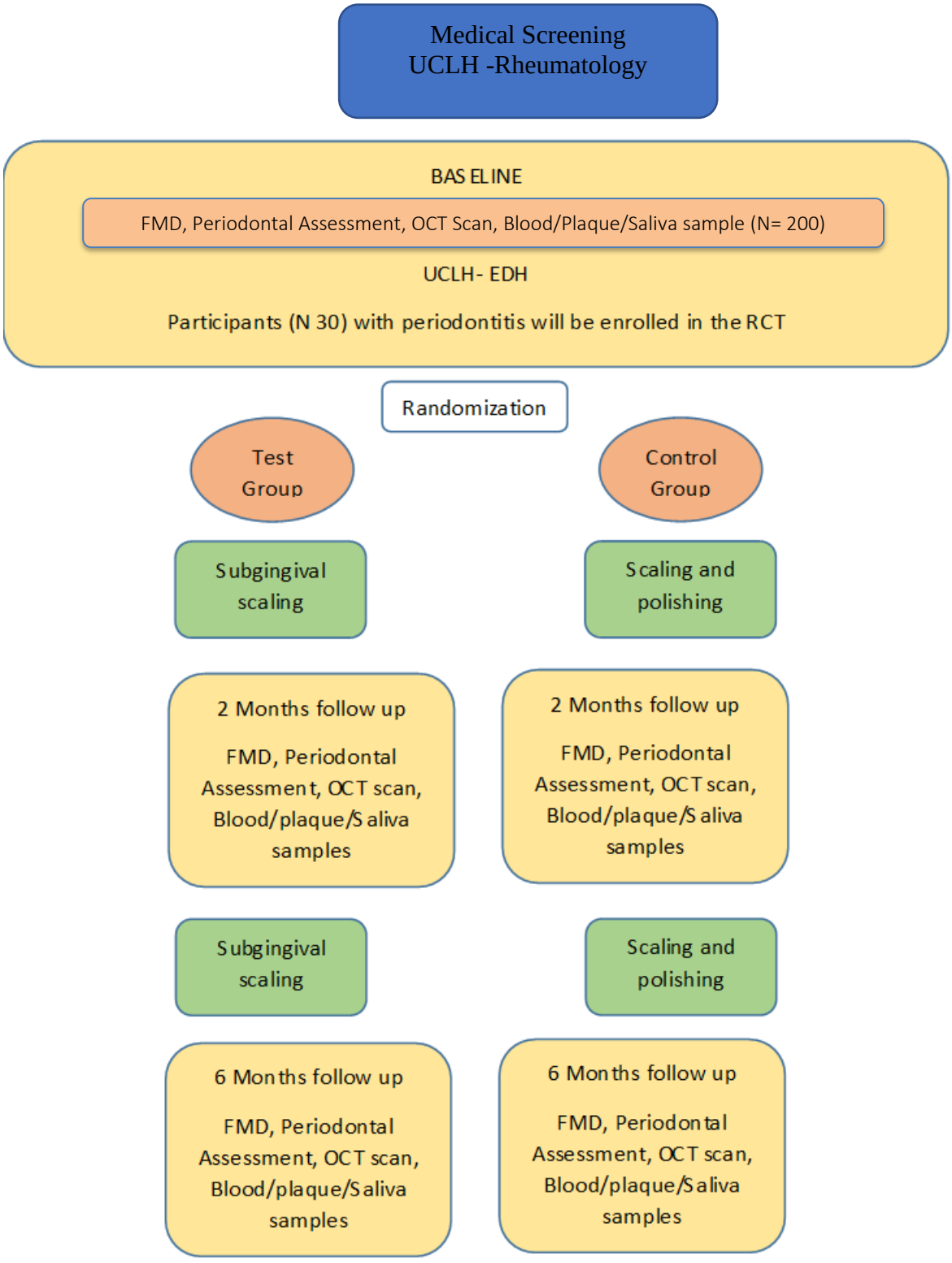
The Investigator will explain that participants are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

No trial procedures will be conducted prior to the participant giving consent by signing the Consent form. Consent will not denote enrolment into trial.

A copy of the signed Informed Consent form will be given to the participant. The original signed form will be retained in the site file and a copy placed in the medical notes.

The PIS and consent form will be reviewed and updated, if necessary, throughout the trial (e.g., where new safety information becomes available) and participants will be re-consented as appropriate.

Trial procedure



Screening (Visit 1) (Day 0) (30 minutes):

- Signed written consent.
- Demographics, medical history, and concomitant medications recording.

Baseline assessment (Visit 2) - to be scheduled within 4 weeks of the screening visit (130 minutes):

- Demographics, medical history, and concomitant medications recording.
- Orthopantomogram OPG (Full Mouth x-ray) (only for the part 2 patients)
- Comprehensive periodontal examination recording.
- Vascular function assessment.
- Optical Coherence Tomography (OCT) to analyse change in selected skin lesion (only for the part 2 patients).
- QRISK3 questionnaire to assess the cardio-vascular risk.
- Blood samples (2 tablespoons) for the analysis of CRP, dsDNA autoantibodies, kidney, and liver function test), Plaque samples and saliva samples (1 teaspoon)

Periodontal Treatment Visit 3 – to be scheduled within 8 weeks (60-120 minutes) (only for the part 2 patients)

- Periodontal Treatment Visit 3 – To be scheduled within 8 weeks (60-120 minutes) Only for part 2 patients
- Randomization
- Medical history recording
- Full mouth root surface debridement under local anaesthesia (IPT) **(test group)**
- Full mouth scaling and polishing (CPT) (control group).

2 months follow-up (Visit 4) - (130 minutes) (only for the part 2 patients)

- Demographics, medical history, and concomitant medications recording.
- Comprehensive periodontal examination recording.
- Blood samples collection (approx. 2 tablespoons)/Saliva (1 teaspoon) and plaque Samples.
- Vascular function assessment (FMD).
- Optical Coherence Tomography (OCT) to analyse change in selected skin lesion.

Periodontal treatment (Visit 5) - (60 minutes) (only for the part 2 patients)

- Medical history recording
- Full mouth root surface debridement under local anaesthesia (IPT) (test group)
- Full mouth scaling and polishing (CPT) (control group)

6 months follow-up (Visit 6) (only for the part 2 patients)

- Demographics, medical history, and concomitant medications recording, including QRISK3 questionnaire to assess the cardio-vascular risk assessment
- Comprehensive periodontal examination recording.
- Blood samples collection (approx. 2 tablespoons)/Saliva (1 teaspoon) and plaque samples.
- Vascular function assessment.
- Optical Coherence Tomography (OCT) to analyse change in selected skin lesion.
- Study Completion.

Randomization Procedures

It is a single blinded trial, and the vascular technician will be the only one blinded for the study. Patients with SLE and periodontitis who consent to participation will be randomised into 2 groups, total 30 participants, Test group (n=15) & Control group (n=15). Participant randomisation will be undertaken by the trial coordinator at ECIC (Eastman clinical Investigation centre) UCL Eastman Dental Institute using computer-based system after patients' baseline assessments.

Following participant consent, and confirmation of eligibility. The registration/randomisation procedure described will be carried out. The trial coordinator will do the randomisation process and variables for age, lupus severity and smoking. Participants are enrolled into the trial following: consent, confirmation of eligibility, pre-treatment assessments (see section 7), completion of the registration/randomisation process, allocation of the participant trial number and intervention by the central coordinating team/remote system.

INTERVENTION PROCEDURES

Periodontal Procedures

Examiner Calibration

A total of 10 non-study subjects from the staff suffering from moderate periodontitis will be recruited and used for the examination. The designated examiner will measure full mouth PPD and REC (CEJ-FGM distance) for all 10 subjects using a manual, UNC-15 periodontal probe with mm markings. Six sites will be measured for each natural tooth (excluding third molars). PPD and

CEJ-FGM distance measurements will be rounded up to the nearest millimetre, CAL will be then calculated from the formula (PPD minus CEJ-FGM). On the same day (min. 15mins separation) the examiner will evaluate the same subjects for a second time.

Upon completion of all measurements, intra-examiner repeatability for CAL measurement will be assessed. Examiner will be judged to be reproducible after meeting the pre-determined success criteria (the percentage of agreement within ± 2 mm between repeated measurements should be at least 98%).

Comprehensive Periodontal Examination

Clinical periodontal parameters will be performed by one calibrated examiner using a manual University of North Carolina (UNC-15) periodontal probe at six sites per tooth (i.e., mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) on all teeth (excluding 3rd molars) at baseline, 2 months and 6 months follow-up visits.

At proximal sites (mesiobuccal, distobuccal, mesiolingual, distolingual), the probe tip will be placed as close to the interproximal area as possible. On the buccal and lingual surfaces of the tooth, measurements will be made at the mid-buccal, mid-lingual points and the probe shall follow the root contour. Whenever a clinical measure is between two millimetres it will be rounded up. At proximal areas the probe, will be placed against the contact surface and the tip will be as close as possible to the interproximal space. With the periodontal probe in position and after calling the pocket depth reading, the examiner will record the recession (REC) as a positive or negative value if the free gingival margin (FGM) occurs coronal or apical respectively to the cemento-enamel junction (CEJ). In the latter case the examiner will reinsert the probe angled 45° degrees into the site to detect the CEJ. If the CEJ is not detectable for anatomical or restorative reasons the examiner will adopt clinical landmarks as follow:

- Crown Margin (Recorded as Cr)
- Rotated teeth, mid-buccal and mid-lingual readings are taken from the clinical midpoints

Clinical attachment level (CAL) will be calculated from the formula PPD minus REC. Full mouth plaque scores (FMPS) will be recorded as the percentages of total surfaces (6 aspects per tooth), which revealed the presence of plaque. A binary score will be assigned to each surface (1 for plaque present, 0 for absent)[367]. A full mouth bleeding score will be recorded in the same manner.

Periodontal Treatment

After the baseline examination, the test group will undergo 1 session of periodontal therapy consisting of supra- and sub-gingival dental instrumentation (IPT) under local anaesthesia by an experienced periodontist. The control group will receive a sham treatment consisting of supra-gingival scaling and polishing (CPT). Participant in the control group will be examined 2 months and 6 months after baseline examination, if signs of disease progression are detected, treatment outside the study contest will be provided. After 6 months, all the participants will receive treatment if they require so irrespective of any group

Assessment of periodontal disease progression

At 2 and 6 months visits the gum probing depth of the pockets detected in the control group will be compared to the baseline measurements to detect signs of disease progression. If a participant presents 10 or more sites with at least 3 mm of additional probing depth, the subject will exit from the study and treated outside the study contest.

Radiographic examination

A full mouth X-ray (Orthopantomogram/OPG) will be prescribed to the patient to evaluate the signs of bone loss.

Laboratory procedures

Laboratory test	Parameters
BLOOD	
Haematology	Blood cell count, LFT, KFT and Evaluate the systemic inflammatory (hs-CRP, IFN-γ, IL-10, IL12-p40, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, BAFF, APRIL, TGFβ1) and lipidomic, transcriptomic and oxidative profile (d-ROMs) and markers of endothelial activation (E-Selectin, ICAM-3, P-Selectin, Thrombomodulin)

Observations and measurements

Medical History

A complete medical history is to be obtained at the screening visit. The medical history is to include demographic background information and dental status information.

Blood pressure, body weight and height

Blood pressure, height, weight, and waist circumference will be measured during the screening visit. The patient will be screened for cardiovascular risk using QRISK3 questionnaire.

Concomitant medications, procedures, and supportive therapies

All concomitant medications, procedures and supportive therapy will be monitored and recorded throughout the study.

Flow cytometry for B cell panel assessment

We will focus on analysing B-cell subpopulations by multi-parameter flow cytometry using the following markers: CD19 (for circulating B-cells), CD38, IgD (for Bm1-Bm5 subsets), CD27, IgD (for memory B-cell subsets), CD24, CD38 (for T1/T2, memory, mature B-cells and plasma), and BAFF-R, in addition to intracellular cytokine staining (IFNγ, TNFα, IL-2, 4, 6, 10, 12, TGFβ1), using protocols already well-established in our laboratory.

We will also assess the lipidomic and transcriptomic profiles of sorted and bulk PBMCs for SLE patients and controls as per protocols already available in our lab.

SLE disease activity and damage assessment

SLE disease activity will be assessed using validated outcome measures, such as SELENA-SLEDAI and BILAG scores. In addition, the cumulative damage due to SLE will be appreciated using the validated SLICC score.

Optical Coherence Tomography

Optical coherence tomography (OCT) is an optical signal acquisition and processing method. It captures micrometre-resolution, three-dimensional images from within optical scattering media. The use of relatively long wavelength light allows it to penetrate into the scattering medium. OCT (Multi-Beam Swept-source Frequency Domain OCT, Vivo sight, Michelson Diagnostics) will be used to analyse changes in a selected skin lesion related to SLE (if present).

Blood Sampling / Saliva Samples/Plaque samples

Blood samples will be collected by single venepuncture from patient's arm complying with the standard procedure of the local hospital. 2 EDTA vacutainer (Pink top, 10 ml) tubes, 1 Citrate vacutainer tube (Light Blue top, 6ml) and 1 plain tube (Red top, 6ml) will be collected and processed for serum and plasma separation within 1 hour. Serum and plasma will be stored in

multiple aliquots in a -70° freezer. Saliva samples (1 ml) and plaque sample will also be collected from patients and stored at -70° freezer for microbial analysis. samples collected, stored, and analysed for research (DNA extraction) into markers of inflammation relevant to periodontitis and/or other inflammatory diseases

Sample Storage and Transfer

In the study, saliva, plaque, and blood samples will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them.

The saliva, plaque and blood samples will be appropriately sent to UCLH, Rheumatology department lab 4th floor, Rayne's Building, 5 University Street, London, WC 1E 6JF for biomarker analysis. The samples will be processed, stored, and disposed in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004.

The saliva, plaque and blood samples will not be transferred to any party not identified in this protocol and are not to be processed and/or transferred other than in accordance with the patients' consent. At the end of the study, any samples left over will be kept for 5 years and either used for further research (pending ethical approval) or destroyed.

Flow-mediated dilatation

All measurements of endothelium-dependent flow-mediated dilatation (FMD) in this study will be collected from the right brachial artery by high-resolution ultrasound imaging (Acuson XP10 7-MHz linear probe and automated vessel-diameter measurements, Brachial Tools, MIA) and will be performed in a temperature-controlled room after 10 minutes' rest and laying supine on a bed. Endothelium-independent vasodilatation will be also assessed following a 25µg dose of GTN administered sublingually. Doppler-derived flow measurements (using a pulsed-wave Doppler signal at a 70° angle) will also be obtained continuously.

Discontinuation or withdrawal of participants

Reasons for withdrawal

Subjects will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw subjects from the study for any of the following reasons:

- Inter-current illness.

- Subject request.
- Protocol violations.
- Administrative reasons.
- Failure to return for follow-up.
- General or specific changes in the subject's condition unacceptable for further treatment in the judgment of the investigator.

Completion/Withdrawal Procedures

At the time of withdrawal, all study procedures outlined for the end of study visit should be completed. The primary reason for a subject's withdrawal from the study is to be recorded. Identifiable data or tissue already collected with consent will be retained and used in the study. No further data or tissue will be collected, or any other research procedures carried out on or in relation to the participant.

Trial Termination

This study may be prematurely terminated, if in the opinion of the investigator there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the investigator by the terminating party.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Failure to enter subjects at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data

Definition of end of trial

The expected duration of the trial is 6 months from recruitment of the first participant and the end of trial is the date of the last visit of the last participant

Recording and reporting adverse effects

Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or trial participant, which does not necessarily have a causal relationship with the intervention involved.
Serious Adverse Event (SAE).	Any adverse event that: 8 results in death, 9 is life-threatening*, 10 requires hospitalisation or prolongation of existing hospitalisation**, 11 results in persistent or significant disability or incapacity, or 12 consists of a congenital anomaly or birth defect.
* A life- threatening event, this refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. **VIII. ** Hospitalization is defined as an in-patient admission, regardless of length of stay. Hospitalization for pre-existing conditions, including elective procedures do not constitute an SAE.**	

Assessments of Adverse Events

Each adverse event will be assessed for severity, causality, seriousness, and expectedness as described below.

Severity

The generic categories below.

Category	Definition
Mild	The adverse event does not interfere with the participant's daily routine, and does not require further intervention; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the participant's routine, or requires further intervention, but is not damaging to health; it causes moderate discomfort

Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

Casualty

The assessment of relationship of adverse events to the intervention is a clinical decision based on all available information at the time of the completion of the case report form.

If a differentiated causality assessment which includes other factors in the trial is deemed appropriate, please add/amend the following wording to specify:

It is of particular importance in this trial to capture events related to the procedure (specify e.g., surgery) / product failure / mandatory concomitant medications / device(s) (refer to section 9.17 for reporting requirements). The assessment of relationship of an adverse event to this/these additional safety issue(s) will also be carried out as part of the trial.

The differentiated causality assessments will be captured in the trial specific CRF/AE Log and/or SAE form.

The following categories will be used to define the causality of the adverse event:

Category	**Definition**
Definitely:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Possibly	There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial intervention). However, the influence of other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events).
Unlikely	There is little evidence to suggest there is a causal relationship (e.g., the event did not occur within a reasonable time after administration of the trial intervention). There is another reasonable explanation for the event (e.g., the participant's clinical condition, other concomitant treatments).
Not related	There is no evidence of any causal relationship.
Not Assessable	Unable to assess on information available.

Expectedness	
Category	Definition
Expected	An adverse event which is consistent with the information about the intervention listed in the SPC, manual of Operation (amend as appropriate) **or clearly defined in this protocol.**
Unexpected	**An adverse event which is not consistent with the information about the intervention listed in the SPC, manual of Operation (amend as appropriate)*** or clearly defined in this protocol.
* This includes listed events that are more frequently reported or more severe than previously reported. The reference document to be used to assess expectedness against the Intervention.	

Recording adverse effects

An adverse event includes any noxious, pathological, or unintended change in anatomical physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory changes occurring in any phase of the clinical study, whether associated with the study or not and whether or not considered drug related. This includes exacerbation or pre-existing conditions or events, intercurrent illnesses, drug interaction or the significant worsening of the disease under study. All adverse events occurring after the start of the study must be reported. Subject entry into the study is defined as the time at which the informed consent is obtained. The nature of each individual adverse event, date and time of onset, duration, severity and relationship to treatment, corrective treatment and change in dosing schedule, must be established by the investigator, and recorded in the CRF.

Assessment of Severity

Severity should be assigned according to the following scale:

MILD: An adverse event easily tolerated, causing a minimal discomfort, and not interfering in daily activities.

MODERATE: An adverse event sufficiently discomforting to interfere with normal activities.

SEVERE: An adverse event preventing normal activities.

Assessment of Causality

Causality should be assigned by the investigator according to the following categories:

DEFINITELY RELATED: An adverse event can be scored as definitely related based on the following:

- known pharmacology of the drug
- a positive challenge
- a reasonably close relationship in time to dosing
- previous reported identical/similar reaction
- toxic levels of the drug
- positive results in a drug sensitivity test

PROBABLY RELATED: An adverse event can be scored as probably related based on the following:

•a reasonable temporal sequence from administration of the drug

•excluding other possible factors, such as underlying disease, concomitant treatment.

POSSIBLY RELATED: An adverse event can be scored as possibly related if it follows a reasonable temporal sequence from administration of the drug that cannot exclude the possibility, although other factors such as underlying disease or concomitant treatment are presumable.

NOT RELATED: An adverse event that does not follow a reasonable temporal sequence from administration of the drug, or that can be reasonably explained by other factors.

Serious Adverse Events (SAEs)

A serious adverse event is any event which is fatal, life threatening, disabling, incapacitating or results in hospitalization, or prolongs a hospital stay or is associated with a congenital abnormality or overdose.

Serious Adverse Event Definitions

LIFE THREATENING EVENT: An adverse event is life threatening if the subject was at immediate risk of death from the event as it occurred: i.e., it does not include a reaction that had it occurred in a more serious form, might have caused death.

DISABILITY/INCAPACITATING EVENT: An adverse event is incapacitating or disabling if the event results in a substantial and/or permanent disruption of the participants' ability to carry out normal life functions.

Reporting of Serious Adverse Events

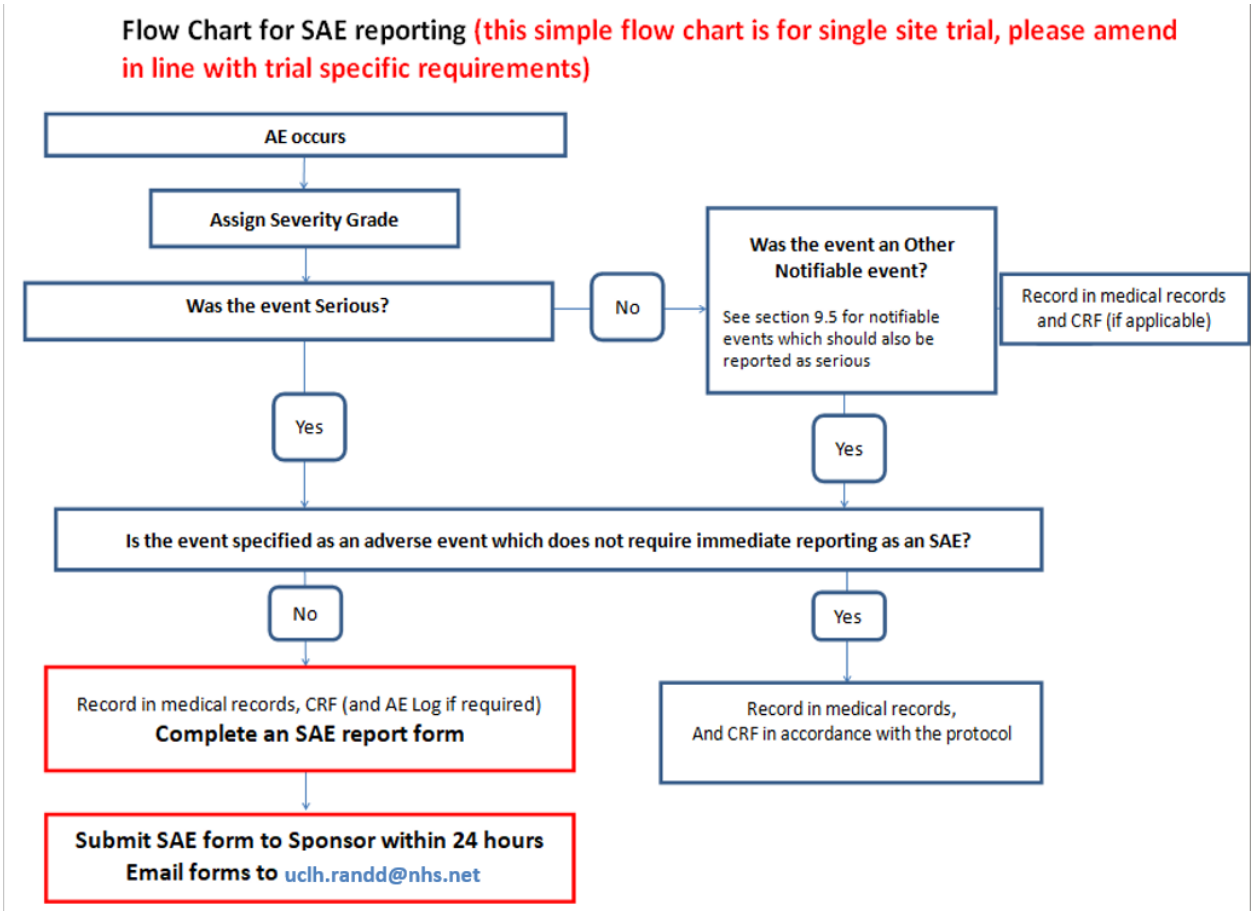
Because of the need, as described below, to report to health authorities all serious adverse events in a timely manner, it is vitally important the investigator report immediately any adverse events

which by the above definitions would be considered serious. Any serious adverse event (including death) due to any cause, which occurs during this clinical study, whether or not related to the study drug, must be reported immediately by telephone to the Principal Investigator or its designee.

Where the event is unexpected and thought to be related to the intervention, this must be reported by the Investigator to the Health Research Authority within 15 days.

Completed SAE forms must be sent within 5 working days of becoming aware of the event to the Sponsor

Email forms to uclh.randd@nhs.net



Reporting Urgent Safety Measures

If any urgent safety measures are taken the CI/ PI shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC and Sponsor of the measures taken and the circumstances giving rise to those measures.

Notification of reporting protocols violation

A reportable protocol violation is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial.

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase.

Trust incidents and near misses

An incident or near miss is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a. It is an accident or other incident which results in injury or ill health.

b. It is contrary to specified or expected standard of patient care or service.

c. It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.

d. It puts the Trust in an adverse position with potential loss of reputation.

e. It puts Trust property or assets in an adverse position or at risk.

Incidents and near misses must be reported to the Trust through DATIX as soon as the individual becomes aware of them.

A reportable incident is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a) It is an accident or other incident which results in injury or ill health.
b) It is contrary to specified or expected standard of patient care or service.
c) It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d) It puts the Trust in an adverse position with potential loss of reputation.
e) It puts Trust property or assets in an adverse position or at risk of loss or damage.

DATA MANAGEMENT

Subject Confidentiality

From the point of enrolment, all case report forms will be completed using subject numbers only. The UCL database (IDHS) Data safe heaven storage and N: drive will be used. The list of study participants will be kept in a password protected computer with a backup copy stored in a locked research cabinet accessible only by research personnel. All source documents will be stored in locked research cabinets located in a secure area within the Eastman Clinical Investigation Centre. All study data to be analysed will be entered in anonymous form using participant number only.

All arrangements should be done to respect confidentiality of personal data of the included participants and meet the requirements of the Data Protection Act. In order to maintain subject privacy, all CRFs, study reports and communications will identify the subject by initials and the assigned subject number. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Completion of Case Report forms

Case report forms will be completed for each study subject. It is the investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, adverse events, and subject status. The investigator, or designated representative, should complete the CRF pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. The investigator must sign and date the Investigator's Statement at the end of the CRF to endorse the recorded data.

Data Handling

Anonymised personal data will be collected from patients in accordance with the patient consent form, patient information sheet and sections of this protocol.

The Anonymised personal data will be appropriately sent to Departmental Statistician Dr. Jacopo Buti (Department of periodontology, Eastman dental Institute) for he will act as the data protection team of such data for the study. All data will be loaded in the appropriate software for analysis.

The archiving of the data will hold at UCL Eastman dental off site archive system and facility for research record storage. Address: Belnor house 4 Fulmar way, Shortgate trading estate, Wickford. They will process, store and dispose of in accordance with all applicable legal and regulatory requirements, including the General Data Protection Regulation (GDPR) and Data

Protection Act 2018 and any amendments thereto. The Eastman investigation centre is equipped with locked storage cabinets for the storage of all study records. All computers are password protected and spread sheets containing any personal data are encrypted.
The Anonymised personal data will not be transferred to any party not identified in this protocol and are not to be processed and/or transferred other than in accordance with the patients' consent.

Statistical considerations

Sample size estimation

Since this is a pilot randomised control trial there is no sample size calculation. Patient with SLE will be continuously screened in order to recruit 30 patients with SLE and PD.

Randomisation Methods

- 30 total participants (n=15) for test group and (n=15) for control group.
- Minimisation randomisation will be used for randomization method.
- Minimization variables include age, smoking and lupus severity
- Use of equal allocation between treatment arms (15 in each arm)
- Clinical trial coordinator will be generating allocation lists and minimization program by computer-based system.

Statistical plan

A total of 30 patients will enter a pilot randomised controlled trial. Individuals enrolled into the study will be randomly assigned to the test or control group in a 1:1 ratio. Allocation to treatment will be concealed in an opaque envelope and revealed to the therapist only. All participants randomized to test, or control will be included in final analyses. Analysis will be performed using last observation carrying forward and as intent to treat population. Secondarily, per protocol analysis will also be performed. Data will be entered in an electronic spreadsheet and checked/proofed for any errors. Continuous data will be displayed as mean and standard deviation; categorical values are as percentages.
All data will be loaded in the appropriate software for analysis. Changes in FMD (primary outcome), circulating biomarkers and intracellular/mitochondrial oxidative stress (secondary outcomes) will be analysed with analysis of variance for repeated measures using a conservative F-test (Greenhouse-Geisser correction). If a treatment by time interaction will be found, pair-wise

comparisons will be performed (Bonferroni–Holm adjustment). Side effects and safety data will be summarized using standard descriptive statistics. Significance will be set to be at $p < 0.05$.

To detect differences in the prevalence of periodontitis between groups Mann–Whitney U tests will be applied. Appropriate corrections (i.e., Bonferroni) will be applied when multiple comparisons will be performed. All secondary outcomes will be analysed by means of multiple linear regression models using age, gender, ethnicity, body weight and SLE assessment as covariates. Correlation analyses with Spearman Rank testing will be performed between all clinical periodontal parameters and systemic continuous variables including vascular function, systemic inflammatory biomarkers and SLE continuous assessments. Alpha value will be set at 0.05 and all statistical analyses will be performed using STATA 15 (Stata Corp LP, College Station, TX, USA).

Record keeping and archiving

At the end of the trial, all essential documentation will be archived securely by the CI for a minimum of 3 years from the declaration of end of trial.

Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with all applicable regulatory requirements.

The sponsor will notify sites when trial documentation can be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request. Custodian for the data generated by the study will be Professor Francesco D`Aiuto, head of unit of Periodontology, Eastman Dental Institute.

Oversight Committees

There is a Trial Management Group (TMG), Trial Steering Committee (TSG) and (TMG), Data Monitoring Committee (DMC) for this study.

The role of the TSC is to provide overall supervision of the trial. The TSC will review the recommendations of the (Independent) Data Monitoring Committee and, on consideration of this information, recommend any appropriate amendments/actions for the trial as necessary. The TSC acts on behalf of the funder(s) and Sponsor.

The role of the DMC is to provide advice on data and safety aspects of the trial but where not all members are independent. Meetings of the Committee will be held every month to review interim analyses, or as necessary to address any issues.

Trial management group (TMG)

TMG consist of Chief Investigator Dr. Coziana Ciurtin, Co-Investigators include, Dr. Marco Orlandi, Dr. Syed Basit Hussain, and Professor Francesco D'Aiuto. and Statistician Dr. Jacopo Buti. The TMG will be responsible for overseeing the trial. The group will meet regularly once a month for six months and will send updates to PIs. The TMG will review recruitment figures, SAEs and substantial amendments to the protocol prior to submission to the REC. All PIs will be kept informed of substantial amendments through their nominated responsible individuals.

- **Trial steering committee (TSG)**

TSG consists of Prof. David Isenberg (world expert in lupus) and Prof. Elizabeth Jury (expert immunologist in lupus). The committee will meet every 6 months or as required based on DMC recommendation.

- **Data monitoring committee (DMT)**

DMT consists of Chief Investigator Dr. Coziana Ciurtin, Co-Investigators include, Dr. Marco Orlandi, Dr. Syed Basit Hussain and Professor Francesco D'Aiuto, and Statistician Dr. Jacopo Buti and the group will review the data every 3 months or as required. As this interventional trial involves a standardised treatment procedure, there is no need for an independent data monitoring committee.

Ethical requirements and patient and public involvement

Ethics

The sponsor will ensure that the trial protocol, participant information sheet, consent form, GP letter and submitted supporting documents have been approved by the appropriate research ethics committee, prior to any participant recruitment. The protocol, all other supporting documents including and agreed amendments, will be documented and submitted for ethical and regulatory approval as required. Amendments will not be implemented prior to receipt of the required approval(s).

Before any NHS site may be opened to recruit participants, the Chief Investigator/Principal Investigator or designee must receive NHS permission in writing from the Trust Research & Development (R&D). It is the responsibility of the CI/ PI or designee at each site to ensure that all subsequent amendments gain the necessary approvals, including NHS Permission (where required) at the site. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The chief investigator will prepare the APR.

Within 90 days after the end of the trial, the CI/Sponsor will ensure that the main REC is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.
The CI will supply the Sponsor with a summary report of the trial, which will then be submitted to the REC within 1 year after the end of the trial.

Patient and public involvement (PPI)
None of the patient or public involvement has been observed.

Monitoring
The sponsor will determine the appropriate level and nature of monitoring required for the trial. Risk will be assessed on an ongoing basis and adjustments made accordingly.
The degree of monitoring will be proportionate to the risks associated with the trial.
A trial specific oversight and monitoring plan will be established for studies. The trial will be monitored in accordance with the agreed plan.
The study has been classified as high medium thus the following plan has been established.
Each site to email the sponsor twice yearly:
1. Delegation log
2. Adverse Event log
. Deviation log
4. Minutes of Trial Steering Committee (or equivalent).
5. Annual progress report (Lead site only) when sent to Ethics Committee.

Finance
The pilot study has been planned in collaboration with the Dept of Rheumatology (Dr Coziana Ciurtin) at UCL/UCLH and we have so far submitted three proposals for funding (two of which have been refused, whilst the last one has been submitted to the UCL Therapeutic Acceleration Support (TAS) Fund). Funding secured from Versus arthritis centre grant for the time period of 1-04-2018 to 31-03-2023. Departmental funds from both Periodontology and Rheumatology Units have been set aside to facilitate the start of the study. Budget code: 539062-100-156780.

Insurance

University College London holds insurance against claims from participants for injury caused by their participation in the trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this trial shall provide negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

Publication policy

The study data will be published or presented in peer reviewed journal, conference presentations and PhD thesis. A process and timeframe for approving and submitting reports for dissemination will be one year after completion of the study.

All proposed publications will be discussed with and reviewed by UCL prior to publishing other than those presented at scientific forums/meetings.

Intellectual property

All background intellectual property rights (including licences) and know-how used in connection with the study shall remain the property of the party introducing the same and the exercise of such rights for purposes of the study shall not infringe any third party's rights. All intellectual property rights and know-how in the protocol and in the results arising directly from the study but excluding all improvements thereto or clinical procedures developed or used by each participating site, shall belong to UCLH. Each participating site agrees that by giving approval to conduct the study at its respective site, it is also agreeing to effectively assign all such intellectual property rights ("IPR") to UCL and to disclose all such know-how to UCL. Each participating site agrees to, at the request and expense of UCL execute all such documents and do all acts necessary to fully vest the IPR in UCL.

Nothing in this section X.Y shall be construed so as to prevent or hinder the participating site from using know-how gained during the performance of the study in the furtherance of its normal activities of providing or commissioning clinical services, teaching and research to the extent that such use does not result in the disclosure or misuse of confidential information or the infringement of an intellectual property right of UCL. This does not permit the disclosure of any of the results of the study, all of which remain confidential.

Schedule of Assessments	**Screening (Pre-treatment assessment)**	**Intervention phase for part 2 study**					**Final visit**
Visit No:	1	2	3		4	5	6
	Visit 1	Visit 2	Visit 3a Control group	Visit 3b Treatment group	Visit 4	Visit 5	Visit 6
Window of flexibility for timing of visits:			e.g. +/- 2 days	e.g., +/- 2days	e.g., +/- 3 days	e.g., +/- 3 days	e.g., +/- 3 days
Informed Consent	X						
Medical History	X	X	X	X	X	X	X
Vital Signs/demographic history	X	X			X		X
Periodontal screening	X						
Periodontal comprehensive Examination		X			X		X
Blood sample (2 Tablespoons), Plaque, and saliva (1 teaspoon) collection		X			X		X
Vascular function assessment (FMD)		X			X		X
QRISK3 to assess CVD risk		X					X
Orthopentomogram OPG		X					
Optical Coherence Tomography (OCT)		X			X		X
Periodontal treatment (Root surface debridement) under LA for test group (IPT)			X	X		X	
Periodontal treatment for control group (scaling and polishing/CPT)			X			X	
Randomisation			X				
Adverse Events review	X	X	X	X	X	X	X
Concomitant Medication review	X	X			X		X

8.4 Appendix 4: Ethical approval for the Randomized Control Trial

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Coziana Ciurtin
Senior clinical lecturer/ Honorary consultant in Rheumatology
UCLH
Rheumatology department
3rd Floor Central, 250 Euston Road
NW1 2PG

Email: hra.approval@nhs.net

22 January 2020

Dear Dr Ciurtin

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**TREATMENT OF PERIODONTAL DISEASE IN SYSTEMIC LUPUS ERYTEMATOSUS. A PILOT RANDOMIZED CONTROLLED CLINICAL TRIAL(student study)**
IRAS project ID:	**250262**
REC reference:	**19/LO/1976**
Sponsor	**UCL**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report

(including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **250262**. Please quote this on all correspondence.

Yours sincerely,
Juliana Araujo

Approvals Specialist

Email: hra.approval@nhs.net

Copy to: *Miss Delasi Apraku*

8.5 Appendix 5: Participant Information sheet (PIS) Consent form, Letters to practitioners

8.5.1 Participant Information Sheet (PIS)

Principal Investigator: Dr Coziana Ciurtin
IRAS ID: 250262
REC Reference:19/LO/1976

PARTICIPANT INFORMATION SHEET

TREATMENT OF PERIODONTAL DISEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS. A PILOT RANDOMISED CONTROL CLINICAL TRIAL (STUDENT STUDY)

Please read this document carefully. Please ask if you do not understand or would like more information.

1. Invitation to participate

You are getting this invitation because you want to be a part of our research. Read the information so that you can make up your mind about participating in this study. Feel free to discuss with family and friends and ask us if there is anything that is not clear or if you would like to know more.

2. Background and purpose

Systemic Lupus Erythematous (SLE) is an auto-immune condition affecting the cells of immune system that are responsible in protecting the body against any disease. The symptoms are fever, pain in chest, swollen joints, hair loss, lack of energy and rashes on face. It is more common in females then males. On the other hand, gum disease is a long-term condition affecting the gums and tissues around the teeth.
SLE and gum diseases are strongly linked, but there are not many studies on this topic. Hence, this study aims at finding out more about this relationship.

3. Is my participation voluntary?

Only you can decide to participate or not. No one else can. After you have made up your mind, a form will be given to you to sign for permission. Even after signing the form if you change your

mind, you can leave, and nobody will ask anything from you. But sometimes your participation in the study cannot continue and the researchers will inform you about it.

4. What are the requirements for taking part?

You must be of 18 years old to take part in this study. Before participating, read the form and sign for permission. After that you will have to inform your GP/dentist for your participation in the research. Make sure you are available for up to 24 weeks which is the duration of the study in case you have gum disease.

5. What will happen to me if I decide to take part?

Firstly, you will sign the consent form. Your first visit is going to be a screening appointment in which we will check your gums, take samples of your saliva, dental plaque and blood. A non-invasive ultrasound scan of your arm will be performed to check the health of your blood vessels. Also, a questionnaire will be given to you. It will ask questions about your oral health (why you go to your dentist). If you are found to not have gum disease, this will be the only study appointment you will attend. If you are found to have active gum disease and are selected, you will be randomly allocated to one of two treatment groups and get treatment for your gum problems. 30 participants with SLE and gum disease will be selected. You might be one of them. In 6 months, you will have to make 6 visits. All study visits will be conducted at the Eastman Dental Hospital.

Visit 1 – Baseline (130 minutes):

At your first visit, you can ask questions if you have any. You will have to sign two copies of the consent form. We will ask you questions about your general health and examine your mouth to confirm you can take part in the rest of the study.

Also, we will do some tests to see your health.

- Whole teeth x-ray which is called Orthopantomogram OPG
- A non-invasive scan of your skin which is called Optical Coherence Tomography (OCT)
- Samples of your blood, saliva and dental plaque
- To see the function of your blood vessels, we will do a Blood vessel Scan (non-invasive ultrasound) (FMD)
- To check your heart disease risk, a questionnaire will be given to you which is called QRISK3.

Visit 2 – Periodontal treatment visit (60 to 120 minutes) within 4 weeks from visit 1

After your visit number 1, you will be placed at random either in a test group or control group.

- At this visit, we will ask you about your health for any changes and we will quickly examine your mouth.
- If you are placed in Test Group, then we will intensively clean your teeth, using local anaesthetic and this will happen in a single visit. You will be also given information about brushing your teeth better.
- If your placed in the Control Group, then we will perform scale and polish of all your teeth in one visit. You will be also given information about brushing your teeth better.
- At the end of the study, we will perform any additional treatment you need anyway.

Visit 3 – 2 Month follow-up (130 minutes)

At this visit, we will ask you about your health for any changes and we will examine your mouth in detail as we did at Visit 1. Also, we will do some tests including:

- A non-invasive scan of your skin (OCT)
- Samples of your blood, saliva and dental plaque
- A blood vessel scan (non-invasive ultrasound) (FMD)

Visit 4 Periodontal treatment (60 minutes)

At this visit, we will ask you about your health for any changes and we will quickly examine your mouth.

- If you are placed in the Test Group, we will repeat the intensive cleaning of your teeth, using local anaesthetic.
- If your placed in the Control Group, then we will perform scale and polish of all your teeth in one visit.

Visit 5 (6-month Follow-up) (130 minutes)

At this visit, we will ask you about your health for any changes and we will examine your mouth in detail as we did at Visit 1 and 3. Also, we will do some tests including:

- A non-invasive scan of your skin (OCT)
- Samples of your blood, saliva and dental plaque
- A blood vessel scan (non-invasive ultrasound) (FMD)
- A questionnaire to check again your heart risk (QRISK3).

Radiographic examination

If you have gum disease, we will request an X-rays of all your teeth to check the gums and bone levels. X-ray uses radiations, which could cause cell damage that may, after many years or decades, turn cancerous. You should consider that having this X-ray you will be exposed to an equivalent dose of 1day of natural background radiation which is also the same amount of radiation exposure when you take a short airplane flight (1-2 hours).

6. Are there any possible risks from the study procedures?

All the procedures in this study are not risky. You might feel a little uncomfortable when we will examine your mouth or when a blood sample will be collected.
The blood vessel ultrasound scan which we will perform in the study also includes you taking a small amount of a medicine (called GTN) which will relax your blood vessels. This medication could lower your blood pressure and might you a headache but for a short time.
All the data acquired in this trial will be used for research purposes however in case of medically significant findings we will inform your General Practitioner and General Dental Practitioner to investigate further.

7. What are the possible benefits in taking part?

If you take part in this study, your teeth and gums will be examined by a gum specialist. If you have gum disease you will get dental cleaning and gum treatment. You will also help us understand more about the connection between SLE and gum disease.

8. Will my taking part in this study be confidential?

We will inform your dentist about your participation in this study and about any gum or teeth problems that we will find. Your information will be stored in a password protected computer at UCL. Your data will be under an identification number and your name will be kept hidden throughout the study (anonymised).

9. What will happen to the Sample I give?

Your samples will be tested in lab to check the activity of SLE and will be marked with your identification number (not your name or other sensitive details). All the samples will be analyses to find out more about the connection between SLE and gum disease. Any remaining sample of blood or dental plaque will be stored for future research for 5 years and then destroyed.

10. General Data Protection Regulation and Data Protection Act 2018 for health and care research

UCL is the sponsor for this study based in the United Kingdom. We will be using information from you and/or your medical records to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. UCL will keep identifiable information about you for up to 3 years after the study has finished. Your rights to access change or move your information are limited, as we need to manage your information in specific ways for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible.
You can find out more about how we use your information at https:/ /www.ucl.ac.uk/legal-services/ucl-general-data-protection-regulation-gdpr or by contacting: gdpr@ucl.ac.uk
UCL will collect information from you and/or your medical records for this research study in accordance with our instructions.
UCLH will collect information from you and your medical records for this research study in accordance with our instructions.
UCLH site will keep your name, NHS number and contact details confidential and will not pass this information to UCL. UCLH will use this information as needed, to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Certain individuals from UCL and regulatory organisations may look at your medical and research records to check the accuracy of the research study. UCL will only receive information without any identifying information. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.
UCLH site will keep identifiable information about you from this study for 3 years after the study has finished.
UCL will collect information about you for this research study from UCLH. UCLH site will not provide any identifying information about you to UCL. We will use this information for research purpose.
When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

11. Complaints

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Contact details are provided at the end of this information sheet. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this. In the unlikely event that you are harmed by taking part in this study, compensation may be available.

If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence, then you may be able to claim compensation. After discussing with your research doctor, please make the claim in writing to Dr. Coziana Ciurtin is the Chief Investigator for the research and is based at the Rheumatology Department UCL. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Contact for further information?
If you need further information, please contact:

Dr. Coziana Ciurtin
Hon Consultant
Department of Rheumatology Unit, UCLH
c.ciurtin@ucl.ac.uk

Or

The Patient Advice and Liaison Service (PALS)
Ground Floor Atrium
University College Hospital
235 Euston Road
London NW1 2BU
Telephone
020 3447 3042 (24 hour answering machine)
Fax: 020 3447 3094
E-mail **PALS**@uhb.**nhs**.uk.

12. Study results

At annual time points, the data collected will be analysed and an annual report will be generated. At the end of the trial, we will inform you of the key findings of the trial. We will also publish the results of this study in scientific journals, conference presentations and PhD thesis but these will not include any personal data from which you can be identified.

13. Are there any reasons why my participation in this study could be ended?

Your participation in this study is voluntary. You can take as much time as you need to decide upon taking part in this study. You may refuse to participate or may discontinue participation from this study at any time without any negative effects on your care and without needing to give any reason. Your participation might be terminated by the Principal Investigator in case you fail to adhere to the study protocol.

14. Ethical approval of the study

All research in the NHS is reviewed by an independent group of people, called a Research Ethics Committee which is there to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favourable ethical opinion by the London - Hampstead Research Ethics Committee (REC ref: 19/LO/1976).

15. Is there a contact to ask questions regarding this study?

You have the right to ask questions about this study at any time. You will be informed about any change to the study that might concern you. Should you have questions, please contact the clinical trial investigator. Details are given below:

Mr. Syed Basit Hussain
Unit of Periodontology, UCL Eastman Dental Institute, Rockefeller Building, 21 University Street, London, WC1E 6DE
Telephone 07477024924
E-mail **basit.hussain5@nhs**.net

Thank you for taking the time to read this information sheet.
A copy of this information sheet and a signed consent form will be given to you.

8.5.2 Consent Form

NHS
University College London Hospitals
NHS Foundation Trust

CONSENT FORM

TREATMENT OF PERIODONTAL DISEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS. A PILOT RANDOMIZED CONTROLLED CLINICAL TRIAL (STUDENT STUDY)

Please initial each box

- I confirm that I have read and understood the information sheet (version 2, Date:15-03-2021) for the above study and have had the opportunity to consider the information and ask questions, which have been answered to my satisfaction. ☐
- I confirm that I have been given "participant information sheet" which gives all the relevant information of the trial. ☐
- I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
- I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University College London from, regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
- I agree to have my blood (2 tablespoons), saliva (1 teaspoon) for, dental plaque and gum fluid samples collected, stored and analysed for research (DNA extraction) into markers of inflammation relevant to periodontitis and/or other inflammatory diseases. ☐
- I agree to my General Practitioner and general dental practitioner being involved in the study, including any necessary exchange of information about me between my GP/GDP and the research team. ☐
- I agree to waive ownership of all data and samples collected including the right to withdraw data from the study. I agree the study samples may be stored for future analysis subject to ethical review. ☐
- I agree to take part in the above study and understand/agree to provide accurate information and follow study instructions. ☐
- I give permission for researchers to contact me in the future to offer participation in future clinical studies ☐

_____	_____	_____
Name of Participant (Print name)	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

8.5.3 Letter to General Practitioner (GP)

University College London Hospitals
NHS Foundation Trust
The Eastman Dental Hospital
Division of Restorative Dentistry
Department of Periodontology
21 University St, Bloomsbury,
London
WC1E 6DE
New Patient (Referral) Enquiries: 020 3456 2300
Appointment Enquiries: 020 3456 1030
Departmental Secretary: 020 3456 1052
Switchboard: 0845 155 5000/020 3456 7890
Web-site: www.uclh.nhs.uk
EASTMAN CLINICAL INVESTIGATION CENTRE
Enquiries & Appointments: Tel: 020 3456 1276

Department of Periodontology

Consultant Staff:
Kalpesh Patel – Clinical Lead
Dr Francesco D'Aiuto – Academic Lead
Dr Ulpee Darbar
Dr Zahra Hussain
Dr Deborah Bomfim
Professor Ian Needleman

Date:

Dear Colleague,,

Re: First Name Last Name,

Address

This is to inform you that the above-named patient has kindly agreed to participate in the pilot randomised controlled clinical trial "TREATMENT OF PERIODONTAL DISEASE IN SYSTEMIC LUPUS ERYTEMATOSUS" at the Eastman Clinical Investigation Centre - Eastman Dental Hospital. This is a pilot randomised controlled clinical trial investigating the impact of periodontal treatment on the endothelial function in a population of patients suffering from Systemic Lupus Erythematosus and Periodontitis.
This study has been reviewed and given a favourable opinion by (_)– The Research Ethics Committee (REC ref: __).
As part of the study, they will have anthropomorphic data (height, weight, waist-hip ratio), biological samples (e.g., blood, saliva, plaque), periodontal and cardiovascular health data collected. Periodontal treatment will be provided according to the study randomization.

Should you have any queries, comments, or suggestions, please do not hesitate to contact me.

Please find the Participant information sheet for your record.

Yours sincerely,

Francesco D'Aiuto DMD, MClinDent, PhD
Professor/ Hon Consultant
Specialist in Periodontics
Head of Unit of Periodontology

8.5.4 Letter to General Dental Practitioner (GDP)

NHS
University College London Hospitals
NHS Foundation Trust
The Eastman Dental Hospital
Division of Restorative Dentistry
Department of Periodontology
21 University St, Bloomsbury,
London,
WC1E 6DE
New Patient (Referral) Enquiries: 020 3456 2300
Appointment Enquiries: 020 3456 1030
Departmental Secretary: 020 3456 1052
Switchboard: 0845 155 5000/020 3456 7890
Website: www.uclh.nhs.uk
EASTMAN CLINICAL INVESTIGATION CENTRE
Enquiries & Appointments: Tel: 020 3456 1276

Department of Periodontology

Consultant Staff:
Kalpesh Patel – Clinical Lead
Dr Francesco D'Aiuto – Academic Lead
Dr Ulpee Darbar
Dr Zahra Hussain
Dr Deborah Bomfim
Professor Ian Needleman

Date:

Dear Colleague,,

Re: First Name Last Name,

Address

This is to inform you that the above-named patient has kindly agreed to participate in the pilot randomised controlled clinical trial "TREATMENT OF PERIODONTAL DISEASE IN SYSTEMIC LUPUS ERYTEMATOSUS" at the Eastman Clinical Investigation Centre - Eastman Dental Hospital. This is a pilot randomised controlled clinical trial investigating the impact of periodontal treatment on the endothelial function in a population of patients suffering from Systemic Lupus Erythematosus and Periodontitis.
This study has been reviewed and given a favourable opinion by (_)– The Research Ethics Committee (REC ref: __).
As part of the study, they will have anthropomorphic data (height, weight, waist-hip ratio), biological samples (e.g. blood, saliva, plaque), periodontal and cardiovascular health data collected. Periodontal treatment will be provided according to the study randomization.

At the baseline visit, as part of our detailed clinical and radiographic examination, we noted

1) XXX
2) XXX
3) XXXXX
4) XXXXXXX

We would be grateful if you could provide routine dental care for this patient including treatment of XXXXX.

Should you have any queries, comments, or suggestions, please do not hesitate to contact me.
Please find the Participant information sheet for your record.

Yours sincerely,

Francesco D'Aiuto DMD, MClinDent, PhD
Professor/ Hon Consultant
Specialist in Periodontics
Head of Unit of Periodontology

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