The orofacial system in early rheumatoid arthritis and individuals at risk

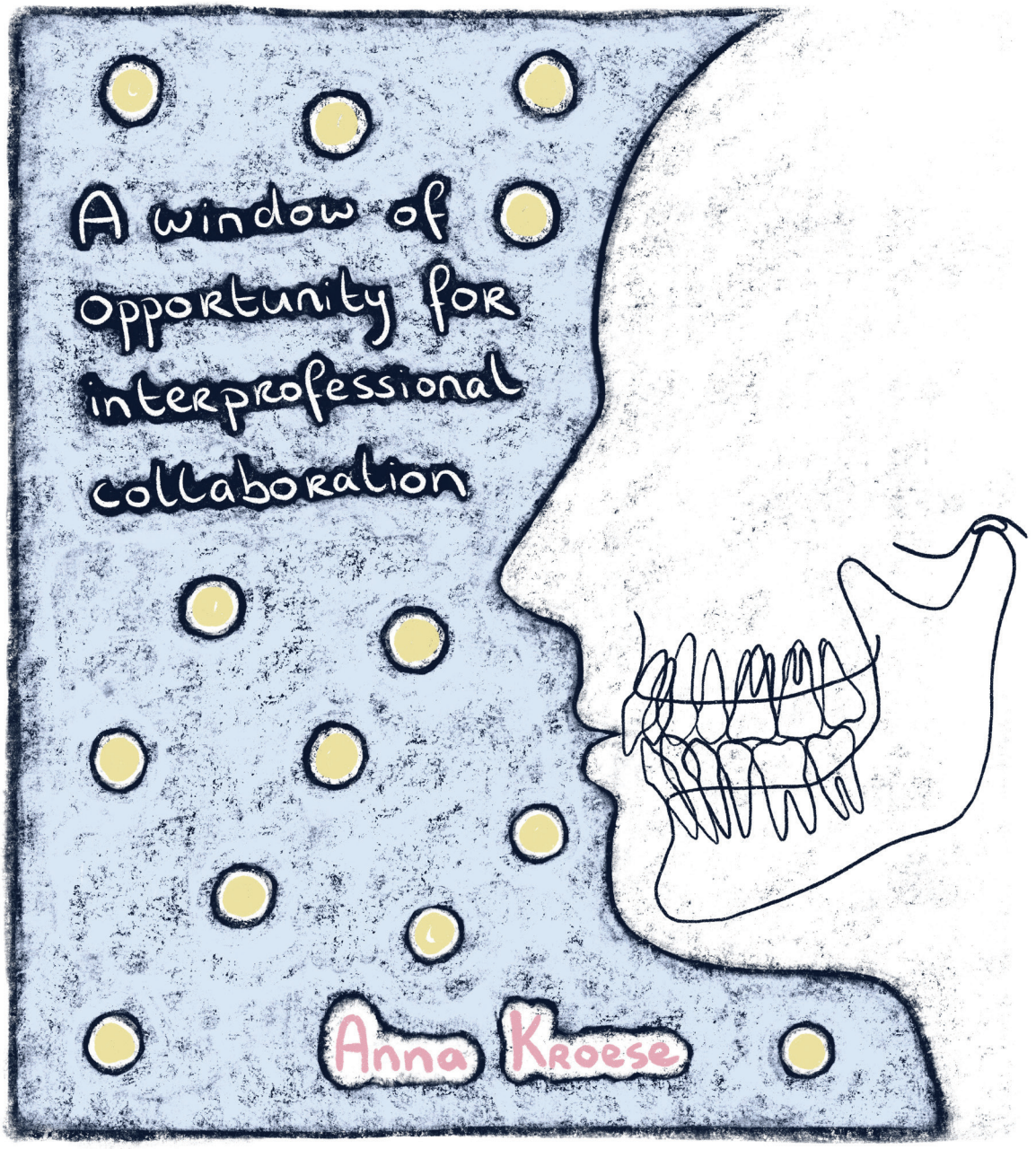
A window of opportunity for interprofessional collaboration

Kroese, J.M.

Publication date
2022

Document Version
Final published version

THE OROFACIAL SYSTEM
IN EARLY RHEUMATOID ARTHRITIS AND INDIVIDUALS AT RISK



The orofacial system in early rheumatoid arthritis and individuals at risk

A window of opportunity for interprofessional collaboration

The orofacial system in early rheumatoid arthritis and individuals at risk

A window of opportunity for interprofessional collaboration

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel

op donderdag 17 februari 2022, te 13.00 uur

door Johanna Margaretha Kroese

geboren te Amsterdam

Publication of this thesis was generously supported by:
Academisch Centrum Tandheelkunde Amsterdam (ACTA).

ISBN: 978 90 830730 1 9

Lay-out and cover design: Anona | www.anonamedia.nl
Printed by: Gildeprint

Promotiecommissie

Promotores:	prof. dr. F. Lobbezoo	Universiteit van Amsterdam
	prof. dr. D. van Schaardenburg	Universiteit van Amsterdam
Copromotores:	dr. C.M.C. Volgenant	Vrije Universiteit Amsterdam
	prof. dr. B.G. Loos	Universiteit van Amsterdam
Overige leden:	dr. G. Aarab	Universiteit van Amsterdam
	prof. dr. P. Alstergren	Malmö University
	dr. B.P. Krom	Universiteit van Amsterdam
	prof. dr. M.L. Laine	Vrije Universiteit Amsterdam
	prof. dr. F.R. Rozema	Universiteit van Amsterdam
	prof. dr. A. Vissink	Rijksuniversiteit Groningen
	prof. dr. R.F. van Vollenhoven	Universiteit van Amsterdam

Faculteit der Tandheelkunde

"A great idea solves multiple problems at the same time."

- Shigeru Miyamoto

TABLE OF CONTENTS

Chapter 1 **General introduction | 9**

Chapter 2 **Study protocol | 23**
Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol
BDJ Open, 2020

Chapter 3 **Temporomandibular joint disorders: prevalence | 41**
Temporomandibular disorders in patients with early rheumatoid arthritis and at-risk individuals in the Dutch population: a cross-sectional study
RMD Open, 2021

Chapter 4 **Temporomandibular joint disorders: consequences | 61**
TMJ pain and crepitus occur early whereas dysfunction develops over time in rheumatoid arthritis
Journal of Oral & Facial Pain and Headache, 2020

Chapter 5 **Temporomandibular joint disorders: treatment | 79**
Corticosteroid injections in the temporomandibular joint temporarily alleviate pain and improve function in rheumatoid arthritis
Clinical Rheumatology, 2021

Chapter 6 **The oral cavity | 97**
Differences in the oral microbiome in patients with early rheumatoid arthritis and individuals at risk of rheumatoid arthritis compared to healthy individuals
Arthritis & Rheumatology, 2021

Chapter 7 **Oral health-related quality of life | 125**
Oral health-related quality of life in patients with early rheumatoid arthritis is associated with periodontal inflammation and painful temporomandibular disorders: a cross-sectional study
Clinical Oral Investigations, 2021

Chapter 8 **General discussion | 143**

Chapter 9 **Summary | 167**
Samenvatting | 171

Chapter 10 **List of publications | 179**
Author contributions | 180
About the author | 183
Dankwoord | 184

General introduction

Chapter 01

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an auto-immune disease affecting the synovial joints. The prevalence of RA in the adult population in developed countries is 0.5-1.0%, [1] and women are two to three times more likely to develop RA than men. [2] In the Dutch population, approximately 17,000 new patients (incidence of 0.1%) are diagnosed with RA each year. [3] Most RA patients suffer from the seropositive form of RA, defined by the presence of specific antibodies: rheumatoid factor (RF) and/or antibodies against citrullinated proteins (ACPA). [4] The American college of rheumatology (ACR) and the European league against rheumatism (EULAR) composed criteria for the classification of RA. This 2010 ACR/EULAR classification system includes several factors, e.g., RF and/or ACPA positivity and symptom duration, while persistent synovitis in at least one joint is required. [5] A method often used for periodical monitoring of RA disease activity is the disease activity score (DAS), based on the clinical examination of 44 joints, blood markers of inflammation, and a subjective evaluation of current general health. To limit patient burden during the examination and increase efficiency, a limited version based on 28 joints, the DAS28, has been developed. [6] In contrast with the DAS44, the DAS28 only monitors the joints in the extremities, since these joints are most often affected.

Because no cure is available for RA, pharmacological treatment focuses on alleviating symptoms and arresting disease progression. [7] Over the last decades, developments in treatment strategies have substantially changed the course of RA, nowadays frequently resulting in remission of the disease. [8,9] Since early intervention results in better clinical outcomes [10] and may prevent joint destruction, [11] there is an important focus on early diagnosis in both research and clinical practice. [2]

Related to this, there also is an increasing interest in identifying individuals at risk of RA. [12] These individuals can be identified, since several characteristics of RA can be present before the clinical outbreak of arthritis, for example arthralgia and increased serum levels of RF and/or ACPA. Although the exact cause of RA development is unknown, consensus exists that multiple factors likely interact, and known risk factors include, amongst others, smoking and obesity. [2] Prediction models for RA development in at-risk individuals based on clinical, serological, and imaging variables have already been developed. [13,14] Although not all predictors are suitable for targeting, interventions focusing on predictors might eventually lead to the prevention of RA in at-risk individuals. [12] Additional predictors, and thus possible targets for prevention, might be present in the

orofacial system, and several interactions between the orofacial system and RA are already acknowledged or subject of interest in current research, as outlined in detail below.

Temporomandibular joint

The temporomandibular joint (TMJ) is a fundamental part of the complex masticatory system, playing a crucial role in mastication and jaw mobility, but also in verbal and emotional expression. [15] Since the TMJ is a synovial joint, it can also be affected by RA, which may even lead to diet modifications due to impaired mastication. [16] The current literature reports a wide range in prevalence of TMJ involvement in RA, from 1.4% to 86%, [17-20] presumably caused by variations in general disease duration, diagnostic criteria, and assessment methods. A review on TMJ bone changes, as diagnosed by cone-beam computed tomography, shows an association between RA and osteoarthritic changes in the TMJ, e.g., erosion, flattening, and sclerosis. [21] Although the need for further confirmation of this relation is pointed out, the importance of monitoring and early detection by health professionals is underlined. However, the transition from the DAS to DAS28 resulted in a decrease in regular consideration of this joint.

To fully explore possible TMJ involvement in RA, all temporomandibular disorders (TMDs) should be considered. TMD is an umbrella term, including pain and dysfunction of the TMJ and the surrounding masticatory muscles, as well as TMJ sounds (i.e., clicking and/or crepitation) during function. TMD pain is considered the most common cause of orofacial pain after dental pain, [22] with a prevalence of 5-8% in the general Dutch population. [23,24] To establish homogeneity in research on TMD, an international consortium composed and validated the research diagnostic criteria for TMD (RDC/TMD), [25] and the later, improved DC/TMD, adapted for use in both research and clinical practice. [26] Research on TMD in RA using the (R)DC/TMD is currently very limited; only a few studies investigated TMD according to the RDC/TMD, of which one on early RA patients, but also including children, and without clear reporting of a total TMD prevalence, [27] and three studies reporting a TMD prevalence ranging from 50% to 91% in RA patients, but with widely varying [28] or unclear disease duration. [29,30]

Bruxism

A common oromandibular movement disorder in humans is bruxism, with a reported prevalence in the general population ranging from 8% to 31%. [31] Bruxism is defined as a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible. These activities can occur during sleep, indicated as sleep bruxism, and during wakefulness, indicated as awake bruxism. [32,33] Bruxism is reported to play a role in the development and maintenance of TMD, and therefore is a relevant factor to consider when analyzing TMD in any population. [34]

Oral cavity

Oral diseases, such as dental caries and inflammation of the periodontal tissues, are initiated by microorganisms in oral biofilms. [35] Timely and adequate removal of dental plaque, a biofilm attached to the hard surfaces of the teeth, by performing oral hygiene is helpful to prevent these oral diseases in most susceptible subjects. Since RA often starts in small joints such as the joints of the hands, [2] causing pain and impaired function, it is no surprise that RA patients report challenges in performing oral self-care. [36] In addition, decreased saliva production was found in RA patients compared to people without RA. [27,37] Furthermore, RA patients have an increased risk of developing secondary Sjögren's syndrome, an auto-immune disease affecting fluid-producing glands, often including the salivary glands. [38] Saliva plays an important role in the protection against oral diseases in several ways, e.g., by containing and preserving the important innate immune cells oral polymorphonuclear neutrophils, [39,40] by its buffer capacity, and by antimicrobial action due to the proteins it contains. [41] Decreased saliva production can thus further complicate maintaining a healthy oral cavity in RA patients. Corresponding to this, the presence of dental plaque and dental caries were found to be higher in RA patients compared to healthy controls, [42,43] and caries prevalence is related to higher disease activity according to the DAS28. [43,44]

Periodontitis

Inadequate dental plaque removal can also lead to inflammation of the gingiva. This may eventually progress to inflammation of the underlying tooth-supporting tissues resulting in periodontitis in susceptible individuals. Periodontitis is a chronic, destructive inflammatory disease, with the severe form affecting approximately 11% of the worldwide adult population, [45] and has been considered a comorbid disorder for several other chronic diseases. [46-48]

Current literature reports common risk factors for periodontitis and RA, such as smoking, and also suggests a bidirectional relationship between both diseases. [49] Literature reviews support a significant association, with an increased relative risk of periodontitis in people with RA compared to healthy controls (relative risk: 1.13), [50] and vice versa an increased relative risk of RA in persons with periodontitis (relative risk: 1.27). [51] Another literature review shows a

significant decrease in RA disease activity according to the DAS28 and systemic inflammatory markers after non-surgical treatment of periodontitis. [52] While in these reviews the limitations of the included studies and the need for further research are pointed out, more recent studies confirm both associations [53-55] and the positive effect of periodontitis treatment on RA disease activity as measured by the DAS28. [56] For the reversed effect, of RA treatment on periodontitis, results are contradictory; while one literature review shows significant improvement of periodontal status after systemic RA treatment, [57] more recent studies show no significant effect of anti-rheumatic treatment on periodontal parameters. [58,59]

Oral microbiome

As previously mentioned, microorganisms play an important role in the development of several oral diseases. With over 700 species, the oral cavity houses the second most diverse microbial community in the human body. Oral microorganisms form communities in biofilms, which are attached to both the dental hard tissues and the soft tissues of the oral mucosa. [60] Formerly, specific species were investigated and linked to oral diseases. However, it is currently more recognized that the oral microbiome is a complex system, and research should focus on possible dysbiosis and shifts in microbiome composition, instead of focusing on specific species. [60] Of particular interest in relation to RA is the possible role of a shift in microbiome composition on disease development in at-risk individuals; the precise mechanism of RA onset is still unknown, but it has been suggested that RA originates at human mucosal sites, such as the gut and oral mucosa, with an important role attributed to the local microbiome. [61,62] Within the oral cavity, there was a particular interest in *Porphyromonas gingivalis* (*P. gingivalis*), a gram-negative, anaerobic bacterium associated with active periodontitis. [63] The interest of the researchers in the field of RA pathogenesis was due to the capability of *P. gingivalis* with its abundant proteolytic enzymes to citrullinate various proteins. Because of this, it was hypothesized that *P. gingivalis* possibly has the capability to trigger ACPA production and initiate an RA-associated immune response. [4] This hypothesis is supported by studies that focus on *P. gingivalis* only, reporting increased levels of antibodies to *P. gingivalis* in RA patients, [64] and RA specific antibodies to human enolase with a possible cross-reactivity to bacterial enolase of *P. gingivalis*. [65] However, more recent studies that analyzed the composition of the oral microbiome in both RA patients and individuals at risk of RA found other genera, including *Prevotella*, to be associated with RA, [66-68] and results on abundance of *P. gingivalis* are inconsistent. [66,67,69] Corresponding to the general trend in research on oral microbiology mentioned earlier, it is thus suggested that future studies on oral microbiology in relation to RA should focus on the microbiome as a whole, rather than focusing on specific bacteria.

Oral health-related quality of life

While objective evaluations provide insight into disease activity and possible clinical consequences, the importance of patient-reported outcome measures (PROMs) is increasingly recognized in RA research and patient care. [70-72] A valuable PROM is health-related quality of life (HRQoL), which can be decreased by pain and functional disability caused by RA. [73] Oral diseases and complaints originating from the orofacial system, such as TMD, caries, and periodontitis, can also negatively influence the QoL. [74-77] The subjective influence of orofacial conditions can be expressed as the OHRQoL (*oral* health-related quality of life). [78] Congruent to the earlier described TMJ involvement and increased prevalence of caries and periodontitis in RA, the current literature reports lower OHRQoL in RA patients compared to healthy controls. [79]

Outline of the thesis

While in general there is an important focus on the timeframe around RA onset, for all aforementioned aspects of the orofacial system, current knowledge is very limited or even absent on individuals in the preclinical and early stages of RA disease. The overall aim of this thesis is therefore to increase knowledge of the orofacial system during this timeframe, i.e., in patients with early RA (ERA) and at-risk individuals. The results may identify areas of interest for interprofessional collaborations in the treatment and management of these specific groups.

Primarily, this was achieved by means of an observational study investigating several orofacial aspects in patients with ERA and at-risk individuals. The key objectives were to investigate prevalence of TMD and periodontal disease, and to describe the composition of the oral microbiome in comparison to healthy controls. To identify the treatment need on a subjective level, OHRQoL was measured and analyzed in relation to several orofacial conditions. An additional objective of the observational study – to investigate possible orofacial predictors for RA development in at-risk individuals – stretches beyond the span of this thesis.

To compare TMJ variables in ERA patients to patients with established RA, an existing database on patients with various general disease durations was used.

This database was also used to investigate the clinical effect of corticosteroid injections in the painful TMJ of RA patients.

Chapters of this thesis
Chapter 1 is the general introduction to this thesis.

Chapter 2 is an extensive description of the study protocol of the observational study that was designed to investigate several orofacial aspects in patients with early rheumatoid arthritis (ERA) and individuals at risk of RA.

Chapter 3 describes findings on the prevalence of temporomandibular disorders (TMD) and bruxism in patients with ERA and individuals at risk of RA compared to a healthy control group. Furthermore, non-familiar pain and non-painful symptoms in the temporomandibular joint (TMJ) and surrounding muscles are reported and discussed.

Chapter 4 reports on clinical TMJ symptoms and inflammatory mediators in TMJ synovial fluid in RA patients. A comparison is made between patients with ERA and patients with established RA.

Chapter 5 explores the clinical value of corticosteroid injections for the treatment of TMJ pain and dysfunction in patients with RA.

Chapter 6 is an extensive description of findings on periodontal health and the oral microbiome of subgingival dental plaque, saliva, and tongue coating of patients with ERA and individuals at risk of RA compared to healthy controls.

Chapter 7 reports on oral health-related quality of life (OHRQoL) in patients with ERA and individuals at risk of RA compared to healthy controls. Results on dental status, periodontal health, xerostomia, and TMD are described, and possible associations with OHRQoL are reported.

Chapter 8 is a general discussion. Recommendations for future research and clinical practice are provided.

References

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376:1094-1108.
2. Littlejohn EA, Monrad SU. Early Diagnosis and Treatment of Rheumatoid Arthritis. Prim Care. 2018;45:237-255.
3. Netherlands Institute for Health Services Research. Tabellenboek Reumatische aandoeningen in Nederland, 2016. Available from: https://www.nivel.nl/sites/default/files/bestanden/Tabellenboek_Reumatische_aandoeningen_Nederland.pdf
4. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nat Rev Immunol. 2017;17:60-75.
5. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-1588.
6. van Riel PL, Renskers L. The Disease Activity Score (DAS) and the Disease Activity Score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol. 2016;34:S40-S44.
7. Arora S, Rafiq A, Meenakshi J. Management of rheumatoid arthritis: Review of current guidelines. J Arth Joint Sur. 2016;3:45-50.
8. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet. 2007;370:1861-1874.
9. Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017;389:2338-2348.
10. Nell VP, Machold KP, Eberl G, Stamm TA, Uffmann M, Smolen JS. Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. Rheumatology (Oxford). 2004;43:906-914.
11. van Dongen H, van Aken J, Lard LR, Visser K, Ronday HK, Hulsmans HM, et al. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum. 2007;56:1424-1432.
12. van Boheemen L, van Schaardenburg D. Predicting Rheumatoid Arthritis in At-risk Individuals. Clin Ther. 2019;41:1286-1298.
13. van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis. 2013;72:1920-1926.
14. Rakieh C, Nam JL, Hunt L, Hensor EM, Das S, Bissell LA, et al. Predicting the development of clinical arthritis in anti-CCP positive individuals with non-specific musculoskeletal symptoms: a prospective observational cohort study. Ann Rheum Dis. 2015;74:1659-1666.
15. Ahmad M, Schiffman EL. Temporomandibular Joint Disorders and Orofacial Pain. Dent Clin North Am. 2016;60:105-124.
16. Orla G, Bechet S, Walshe M. Modified Diet Use in Adults with Temporomandibular Disorders related to Rheumatoid Arthritis: A Systematic Review. Mediterr J Rheumatol. 2020;31:183-189.
17. Byun SH, Min C, Choi HG, Hong SJ. Increased Risk of Temporomandibular Joint Disorder in Patients with Rheumatoid Arthritis: A Longitudinal Follow-Up Study. J

Clin Med. 2020;9:3005.
18. Bono AE, Learreta JA, Rodriguez G, Marcos JC. Stomatognathic system involvement in rheumatoid arthritis patients. Cranio. 2014;32:31-37.
19. Wolfe F, Katz RS, Michaud K. Jaw pain: its prevalence and meaning in patients with rheumatoid arthritis, osteoarthritis, and fibromyalgia. J Rheumatol. 2005;32:2421-2428.
20. Lin YC, Hsu ML, Yang JS, Liang TH, Chou SL, Lin HY. Temporomandibular joint disorders in patients with rheumatoid arthritis. J Chin Med Assoc. 2007;70:527-534.
21. Campos DES, de Araujo Ferreira Muniz I, de Souza Villarim NL, Ribeiro ILA, Batista AUD, Bonan PRF, et al. Is there an association between rheumatoid arthritis and bone changes in the temporomandibular joint diagnosed by cone-beam computed tomography? A systematic review and meta-analysis. Clin Oral Investig. 2021;25:2449-2459.
22. Durham J, Newton-John TR, Zakrzewska JM. Temporomandibular disorders. BMJ. 2015;350:h1154.
23. De Kanter RJ, Truin GJ, Burgersdijk RC, Van 't Hof MA, Battistuzzi PG, Kalsbeek H, et al. Prevalence in the Dutch adult population and a meta-analysis of signs and symptoms of temporomandibular disorder. J Dent Res. 1993;72:1509-1518.
24. Visscher CM, Ligthart L, Schuller AA, Lobbezoo F, de Jongh A, van Houtem CM, et al. Comorbid disorders and sociodemographic variables in temporomandibular pain in the general Dutch population. J Oral Facial Pain Headache. 2015;29:51-59.
25. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord. 1992;6:301-355.
26. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. J Oral Facial Pain Headache. 2014;28:6-27.
27. Crincoli V, Anelli MG, Quercia E, Piancino MG, Di Comite M. Temporomandibular Disorders and Oral Features in Early Rheumatoid Arthritis Patients: An Observational Study. Int J Med Sci. 2019;16:253-263.
28. Kurtoglu C, Kurkcu M, Sertdemir Y, Ozbek S, Gurbuz CC. Temporomandibular disorders in patients with rheumatoid arthritis: A clinical study. Niger J Clin Pract. 2016;19:715-720.
29. Bracco P, Debernardi C, Piancino MG, Cirigliano MF, Salvetti G, Bazzichi L, et al. Evaluation of the stomatognathic system in patients with rheumatoid arthritis according to the research diagnostic criteria for temporomandibular disorders. Cranio. 2010;28:181-186.
30. Mortazavi N, Babaei M, Babaee N, Kazemi HH, Mortazavi R, Mostafazadeh A. Evaluation of the Prevalence of Temporomandibular Joint Involvement in Rheumatoid Arthritis Using Research Diagnostic Criteria for Temporomandibular Disorders. J Dent (Tehran). 2018;15:332-338.
31. Manfredini D, Winocur E, Guarda-Nardini L, Paesani D, Lobbezoo F. Epidemiology of bruxism in adults: a systematic review of the literature. J Orofac Pain. 2013;27:99-110.
32. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, et al. Bruxism defined and graded: an international consensus. J Oral Rehabil. 2013;40:2-4.
33. Lobbezoo F, Ahlberg J, Raphael KG, Wetselaar P, Glaros AG, Kato T, et al. International consensus on the assessment of bruxism: Report of a work in progress. J Oral Rehabil. 2018;45:837-844.
34. Jimenez-Silva A, Pena-Duran C, Tobar-Reyes J, Frugone-Zambra R. Sleep and awake bruxism in adults and its relationship with temporomandibular disorders: A systematic review from 2003 to 2014. Acta Odontol Scand. 2017;75:36-58.
35. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett. 2014;162:22-38.
36. Protudjer JLP, Billedeau C, Hurst K, Schroth R, Stavropoulou C, Kelekis-Cholakis A, et al. Oral Health in Rheumatoid Arthritis: Listening to Patients. JDR Clin Trans Res. 2021:23800844211012678.
37. Silvestre-Rangil J, Bagan L, Silvestre FJ, Bagan JV. Oral manifestations of rheumatoid arthritis. A cross-sectional study of 73 patients. Clin Oral Investig. 2016;20:2575-2580.
38. Patel R, Shahane A. The epidemiology of Sjogren's syndrome. Clin Epidemiol. 2014;6:247-255.
39. Rijkschroeff P, Loos BG, Nicu EA. Oral Polymorphonuclear Neutrophil Contributes to Oral Health. Curr Oral Health Rep. 2018;5:211-220.
40. Rijkschroeff P, Jansen ID, van der Weijden FA, Keijser BJ, Loos BG, Nicu EA. Oral polymorphonuclear neutrophil characteristics in relation to oral health: a cross-sectional, observational clinical study. Int J Oral Sci. 2016;8:191-198.
41. Llena-Puy C. The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal. 2006;11:E449-455.
42. Manzano BR, da Silva Santos PS, Bariquelo MH, Merlini NRG, Honorio HM, Rubira CMF. A case-control study of oral diseases and quality of life in individuals with rheumatoid arthritis and systemic lupus erythematosus. Clin Oral Investig. 2021;25:2081-2092.
43. Almasi S, Karbalaei Sabbagh M, Barzi D, Tahooni A, Atyabi H, Basir Shabestari S. Relationship between clinical and laboratory findings of rheumatoid arthritis patients with their oral status and disease activity. Caspian J Intern Med. 2021;12:22-28.
44. Ayravainen L, Heikkinen AM, Kuuliala A, Ahola K, Koivuniemi R, Peltola J, et al. Activity of rheumatoid arthritis correlates with oral inflammatory burden. Rheumatol Int. 2018;38:1661-1669.
45. Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res. 2014;93:1045-1053.
46. Preshaw PM, Bissett SM. Periodontitis and diabetes. Br Dent J. 2019;227:577-584.
47. Arboleda S, Vargas M, Losada S, Pinto A. Review of obesity and periodontitis: an epidemiological view. Br Dent J. 2019;227:235-239.
48. Sanz M, Marco Del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and cardiovascular diseases: Consensus report. J Clin Periodontol. 2020;47:268-288.
49. Samborska-Mazur J, Sikorska D, Wyganowska-Swiatkowska M. The relationship between periodontal status and rheumatoid arthritis - systematic review. Reumatologia. 2020;58:236-242.

50. Fuggle NR, Smith TO, Kaul A, Sofat N. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis. Front Immunol. 2016;7:80.
51. de Oliveira Ferreira R, de Brito Silva R, Magno MB, Carvalho Almeida A, Fagundes NCF, Maia LC, et al. Does periodontitis represent a risk factor for rheumatoid arthritis? A systematic review and meta-analysis. Ther Adv Musculoskelet Dis. 2019;11:1759720X19858514.
52. Silva DS, Costa F, Baptista IP, Santiago T, Lund H, Tarp S, et al. Evidence-based research on effectiveness of periodontal treatment in rheumatoid arthritis patients: a systematic review and meta-analysis. Arthritis Care Res (Hoboken). 2021. Online ahead of print.
53. Moura MF, Cota LO, Costa AM, Silva TA, Costa FO. Rheumatoid arthritis associated with the occurrence, severity and extension of periodontitis: A case-control study. J Clin Exp Dent. 2021;13:e389-e396.
54. Karapetsa D, Consensi A, Castagnoli G, Petrini M, Tonelli M, Gennai S, et al. Periodontitis in Italian patients with established rheumatoid arthritis: A cross-sectional study. Oral Dis. 2021;00:1-8.
55. Choi YY, Lee KH. Periodontitis as a risk factor for rheumatoid arthritis: a matched-cohort study. Int Dent J. 2021;S0020-6539(21)00006-X.
56. Nguyen VB, Nguyen TT, Huynh NC, Nguyen KD, Le TA, Hoang HT. Effects of non-surgical periodontal treatment in rheumatoid arthritis patients: A randomized clinical trial. Dent Med Probl. 2021;58:97-105.
57. Zamri F, de Vries TJ. Use of TNF Inhibitors in Rheumatoid Arthritis and Implications for the Periodontal Status: For the Benefit of Both? Front Immunol. 2020;11:591365.
58. de Smit MJ, Westra J, Posthumus MD, Springer G, van Winkelhoff AJ, Vissink A, et al. Effect of Anti-Rheumatic Treatment on the Periodontal Condition of Rheumatoid Arthritis Patients. Int J Environ Res Public Health. 2021;18:2529.
59. Rinaudo-Gaujous M, Blasco-Baque V, Miossec P, Gaudin P, Farge P, Roblin X, et al. Infliximab Induced a Dissociated Response of Severe Periodontal Biomarkers in Rheumatoid Arthritis Patients. J Clin Med. 2019;8:751.
60. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, et al. The oral microbiome - an update for oral healthcare professionals. Br Dent J. 2016;221:657-666.
61. Maeda Y, Takeda K. Host-microbiota interactions in rheumatoid arthritis. Exp Mol Med. 2019;51:1-6.
62. Konig MF. The microbiome in autoimmune rheumatic disease. Best Pract Res Clin Rheumatol. 2020;34:101473.
63. Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, et al. Porphyromonas gingivalis: major periodontopathic pathogen overview. J Immunol Res. 2014;2014:476068.
64. Mikuls TR, Payne JB, Reinhardt RA, Thiele GM, Maziarz E, Cannella AC, et al. Antibody responses to Porphyromonas gingivalis (P. gingivalis) in subjects with rheumatoid arthritis and periodontitis. Int Immunopharmacol. 2009;9:38-42.
65. Lundberg K, Kinloch A, Fisher BA, Wegner N, Wait R, Charles P, et al. Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. Arthritis Rheum. 2008;58:3009-3019.

66. Scher JU, Ubeda C, Equinda M, Khanin R, Buischi Y, Viale A, et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. Arthritis Rheum. 2012;64:3083-3094.
67. Lopez-Oliva I, Paropkari AD, Saraswat S, Serban S, Yonel Z, Sharma P, et al. Dysbiotic Subgingival Microbial Communities in Periodontally Healthy Patients With Rheumatoid Arthritis. Arthritis Rheumatol. 2018;70:1008-1013.
68. Cheng Z, Do T, Mankia K, Meade J, Hunt L, Clerehugh V, et al. Dysbiosis in the oral microbiomes of anti-CCP positive individuals at risk of developing rheumatoid arthritis. Ann Rheum Dis. 2021;80:162-168.
69. Mankia K, Cheng Z, Do T, Hunt L, Meade J, Kang J, et al. Prevalence of Periodontal Disease and Periodontopathic Bacteria in Anti-Cyclic Citrullinated Protein Antibody-Positive At-Risk Adults Without Arthritis. JAMA Netw Open. 2019;2:e195394.
70. Strand V, Wright GC, Bergman MJ, Tambiah J, Taylor PC. Patient Expectations and Perceptions of Goal-setting Strategies for Disease Management in Rheumatoid Arthritis. J Rheumatol. 2015;42:2046-2054.
71. Kaneko Y, Takeuchi T, Cai Z, Sato M, Awakura K, Gaich C, et al. Determinants of Patient's Global Assessment of Disease Activity and Physician's Global Assessment of Disease Activity in patients with rheumatoid arthritis: A post hoc analysis of overall and Japanese results from phase 3 clinical trials. Mod Rheumatol. 2018;28:960-967.
72. Van der Elst K, Verschueren P, Stouten V, Pazmino S, De Groef A, De Cock D, et al. Patient-Reported Outcome Data From an Early Rheumatoid Arthritis Trial: Opportunities for Broadening the Scope of Treating to Target. Arthritis Care Res (Hoboken). 2019;71:1566-1575.
73. Matcham F, Scott IC, Rayner L, Hotopf M, Kingsley GH, Norton S, et al. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: a systematic review and meta-analysis. Semin Arthritis Rheum. 2014;44:123-130.
74. Ferreira MC, Dias-Pereira AC, Branco-de-Almeida LS, Martins CC, Paiva SM. Impact of periodontal disease on quality of life: a systematic review. J Periodontal Res. 2017;52:651-665.
75. Naito M, Yuasa H, Nomura Y, Nakayama T, Hamajima N, Hanada N. Oral health status and health-related quality of life: a systematic review. J Oral Sci. 2006;48:1-7.
76. Dahlström L, Carlsson GE. Temporomandibular disorders and oral health-related quality of life. A systematic review. Acta Odontol Scand. 2010;68:80-85.
77. Baniasadi K, Armoon B, Higgs P, Bayat AH, Mohammadi Gharehghani MA, Hemmat M, et al. The Association of Oral Health Status and socio-economic determinants with Oral Health-Related Quality of Life among the elderly: A systematic review and meta-analysis. Int J Dent Hyg. 2021;19:153-165.
78. Slade GD, Spencer AJ. Development and evaluation of the Oral Health Impact Profile. Community Dent Health. 1994;11:3-11.
79. Schmalz G, Patschan S, Patschan D, Ziebolz D. Oral-Health-Related Quality of Life in Adult Patients with Rheumatic Diseases-A Systematic Review. J Clin Med. 2020;9:1172.

Study protocol

Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol

Johanna M. Kroese, Catherine M.C. Volgenant, Dirkjan van Schaardenburg, Bruno G. Loos, Wim Crielaard, Frank Lobbezoo

Published in: BDJ Open. 2020;6:7.
PMID: 32467775.

Chapter 02

ABSTRACT

Objectives/Aims: Rheumatoid arthritis (RA) is an autoimmune disease affecting the joints, including the temporomandibular joint (TMJ). Early diagnosis and treatment can alleviate symptoms and prevent progression. Predictors for disease outcome in individuals at risk for RA are therefore valuable. While limited information is available on the prevalence of TMJ involvement in early RA, previous studies suggest that RA, periodontitis and the oral microbiome are interrelated. Predictive factors for RA development may thus be present in the oral cavity.
Our two aims are: (1) to assess the prevalence of TMJ involvement in early RA, and (2) to investigate the predictive value of oral factors in RA development.

Materials and Methods: We will include 150 individuals in this multi-center, prospective cohort study: 50 patients with early RA, 50 at-risk individuals, and 50 healthy controls. At baseline, the TMJ, periodontal health, and the oral microbiome will be examined. The general health will be followed over time, on four occasions up to three years.

Discussion: Our results will provide insight into the prevalence and clinical characterization of TMJ involvement in early RA. For at-risk individuals, oral factors can be studied as possible predictors for the development of RA.

Background

Rheumatoid Arthritis (RA) is an autoimmune disease affecting the synovial joints that eventually results in the destruction of cartilage and bone. [1] In developed countries, RA affects 0.5%-1.0% of the adult population. Because no cure is available for RA, pharmacological treatment focuses on arresting disease progression and alleviating symptoms. [2] Early intervention results in better clinical outcomes [3] and may prevent joint destruction, [4] which makes early diagnosis desirable.

Most RA patients are affected by the so-called seropositive form of RA, defined by the presence of specific antibodies: IgM rheumatoid factor (IgM-RF) and antibodies against citrullinated proteins (ACPA). [5] Increased serum levels of these antibodies in healthy individuals imply an elevated risk of developing RA. [6] Interestingly though, not everyone having increased IgM-RF or ACPA serum levels develops RA. Since early diagnosis of RA is favorable, additional predictors of RA development in at-risk individuals would be valuable. Prediction models based on clinical, serological, and imaging variables have already been developed. [7,8]

Additional predictors might be present in the orofacial tissues; RA can affect the temporomandibular joint (TMJ), and previous studies suggest that RA, periodontitis, and the oral microbiome are interrelated, as outlined in detail below.

RA and the TMJ

Being a disease affecting the joints, RA can also affect the TMJ, causing disease-related symptoms. [9-11] A prevalence of TMJ disorders (TMD) in patients with RA ranging from 19%-85.7% has been reported. [12-14] The wide range in prevalence is presumably caused by variations in diagnostic criteria and assessment methods. For patients with early RA, only limited data is available on TMD prevalence; based only on pain on palpation, a prevalence of 10.6% has been reported. [15] To our knowledge, no data on TMD is available for individuals with an increased risk of developing RA. Therefore, research on these specific patient groups is needed.

RA and Oral Health

Reduced saliva production is more prevalent in patients with RA than in people without RA. [9] Furthermore, a positive association between RA and periodontitis has been described, [16,17] although other studies did not find this relation. [18-20] The possible role of *Porphyromonas gingivalis* – a bacterium highly associated with periodontitis – in the immunopathogenesis of RA has been of particular interest

due to its capacity to generate citrullinated proteins. [5,21]

An interesting development in research on microbiology is to focus on the microbiome as a whole, grouping species based on their 16S rDNA gene, instead of focusing on specific bacteria. [22] Therefore, it is of importance to investigate the oral microbiome in relation to RA, and the possible association between oral microbiome dysbiosis and disease progression. Of particular interest are individuals at increased risk of developing RA, and the possible biomarkers in the oral microbiome for RA development.

Aim and objectives

The overall aim of the study described in this protocol is to investigate several aspects of the interaction between RA and the orofacial tissues, focusing on patients with early RA and individuals with an increased risk of developing RA.

The primary objective is to determine the prevalence of TMD within these two groups and a healthy control group. The secondary objectives are to investigate the possible predictive value of oral health and oral microbiome on the development and progression of RA, and to explore the dynamics of the microbiological composition of the oral ecosystem of the three groups.

For the primary objective, the hypothesis is that the prevalence of TMD is higher in patients with early RA and patients with an increased risk of developing RA, compared to a healthy control group. For the secondary objectives, the hypotheses are that periodontal inflammation and oral microbiome dysbiosis might contribute to the development of RA and negatively influence the progression of RA, and that the microbiological composition of the oral ecosystem differs between the three groups.

Methods and design

Study design

The study has a multi-center, prospective cohort study design. Participants will be recruited in three different groups; (1) patients who have been diagnosed with early RA, (2) persons with an increased risk of developing RA, and (3) a control group with no autoimmune conditions. A total number of 150 subjects will be recruited, i.e., 50 in each group. Groups 1 and 2 will be recruited at Reade, an out-patient clinic for rheumatology in Amsterdam, while group 3 will be recruited at ACTA, the Academic Center for Dentistry Amsterdam.

Extensive research data will be gathered at baseline, and all examinations will take place at Reade. The development of general health will be followed up over time, on four occasions up to three years after baseline. The length of the follow-up period was determined based on a study on individuals at-risk of developing RA performed at Reade. This study showed that those who developed arthritis, did so after a median (IQR) follow-up of 12 (6-23) months, and the descending curve of arthritis-free survival flattened after a follow-up of 36 months. [7]

Participants

Subjects are eligible for inclusion if they are aged 18 years or older, have a minimum of 12 natural teeth, and are willing and able to give written informed consent in the Dutch language.

For group 1, patients should be diagnosed with RA according to the treating rheumatologist, within the last year, based on the 2010 EULAR RA criteria. [23] For group 2, the increased risk of developing RA has to be determined by increased serum levels of IgM-RF and/or ACPA.

For group 3, healthy subjects will be recruited at ACTA. Potential subjects for the control group will be excluded when they have an autoimmune condition, or when increased serum levels of IgM-RF or ACPA are found during evaluation of a blood sample collected at the baseline visit. Also, students from ACTA and employees of ACTA and Reade will be excluded from participation in this study, to prevent bias caused by above average oral hygiene/self-care in health care providers.

All potential subjects at Reade will be invited by the treating doctor or nurse to participate in the study. After oral consent, they are contacted and thoroughly informed about the study by a dedicated investigator (JMK). Recruitment for the control group at ACTA will start approximately 6 months after the first inclusion at Reade, to be able to match the control group to the groups at Reade for sex and age. Potential subjects at ACTA will be approached and thoroughly informed by the dentist-investigator (JMK). Subjects in all three groups receive at least one week time to consider participation after being thoroughly informed. If a subject is willing to participate, written consent is obtained.

For all study subjects, participation is always voluntary, and declining to participate will not be of any influence on the medical or dental care patients receive at either Reade or ACTA.

Sample size

The primary objective of this study – to assess the prevalence of TMD in patients with early RA or with an increased risk of developing RA – was used to determine

the sample size.

Previous studies have reported a prevalence of TMJ pain in patients with RA ranging between 19% and 85.7%. [12-14] However, because of the wide variations on disease duration, these studies do not provide specific information on prevalence in patients with early RA. A recent study conducted at Reade on TMJ pain in patients with early RA, using pain on palpation as the only outcome measure, reported a prevalence of 10.6%. [15] To our knowledge, no prevalence data is available on individuals at increased risk of developing RA. In comparison, for the general Dutch population a prevalence of TMD from 5% [24] to 8% [25] has been reported. The lack of sufficient data on patients with early RA or an increased risk of developing RA having TMD, complicates an accurate assessment of the required group sizes. Furthermore, we anticipate different outcomes on prevalence of TMD compared to previous studies, because of the more extensive examination of the masticatory system as compared to previous studies, taking into account both arthrogenous and myogenous components, as further described below.

The limited availability of data complicates a reliable sample size calculation. Thus, based on expectations and anticipated feasibility to recruit participants, we estimate that 30-40 subjects per group are required to adequately explore the primary objective and to determine whether it would be realistic to plan a larger (multi-center) study. Because of an anticipated drop-out rate of 20% after three years, the aim is to include 50 subjects in each group.

Outcome variables

Temporomandibular joint disorders

A TMD diagnosis will be determined according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). [26] The combination of a questionnaire and standardized clinical tests is necessary to determine the possible diagnosis or diagnoses.

Prior to the baseline visit, participants are asked to fill in the symptom questionnaire on TMD pain, headache, jaw joint noises, and closed and open locking of the jaw. During the baseline visit, a standardized examination of the TMJs and the masticatory muscles (temporalis and masseter) will be performed, as described in the DC/TMD clinical examination protocol. [27] Tests will be limited to those items of the protocol needed to be able to determine a TMD diagnosis.

It has been reported that dynamic joint tests and static muscle tests can be of additional value for the confirmation of a suspicion on TMD pain. These tests, distinguishing both an arthrogenous and a myogenous component of TMD, result in a higher specificity than palpation alone. [28] Therefore, in addition to the DC/TMD clinical examination protocol tests, which mainly exist of palpation tests, the dynamic and static tests will be performed.

The dentist performing the clinical research (JMK) was trained in performing the described TMJ examinations by a calibrated specialist in TMD.

General Health

Each participant will be asked to fill in a standard medical questionnaire prior to the baseline visit, to be able to track general health and identify possible confounders, such as comorbid conditions and medication use. During the baseline visit, additional questions will be asked about recent use of antibiotics, because of their reported effect on the oral microbiome, [29] and of analgesics, because of the potential masking effect on pain during the performance of the TMD tests and their possible anti-inflammatory characteristics affecting periodontal tissues and microbiome.

Venous blood is collected from all participants in groups 1 and 2 at intake as a standard procedure at Reade, so analyses from these samples can be used for the current study. To determine serum levels of IgM-RF and ACPA in the control group, a blood sample will be collected during the baseline visit.

On four occasions up to three years after baseline, the patient files of participants will be consulted to determine possible changes in the general health. If information in a patient file is insufficient, the participant will be contacted by phone to collect complementary data.

Oral Health

At baseline, the oral condition of all participants is measured for several intra-oral aspects, but particularly the periodontal health by performing a full periodontal examination. Presence or absence of bleeding on probing (BOP), gingival recessions (positive and negative, to also identify pseudopockets) in millimeters, and pocket probing depth (PPD) in millimeters will be measured on six sites for each tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual). Recording of BOP results in a full mouth BOP percentage. The combination of recessions and PPD results in the amount of clinical attachment loss (CAL). Prior to the probing, the amount of dental plaque will be scored according to the Silness-Loë Index [30] (on a scale from 0 to 3, with 0 being “no plaque” and 3 “abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin”) for all teeth on all six previously described sites. The dentist-examiner (JMK) was trained for the periodontal examination by a periodontologist.

In addition to the examination of periodontal health, a visual intra-

oral inspection will be performed to record possible mucosal lesions and the amount of decayed, missing, and filled surfaces of the teeth (DMFS). [31] The main utility of this inspection is the exclusion of possible sources of intra-oral pain as a confounding factor. In case periodontitis and/or overt caries will be found, the participant and the treating dentist will be informed.

Bruxism

At baseline, both subjective and objective bruxism activity will be measured to study a possible mediating role of bruxism in the prevalence and characterization of TMD in the study population. Subjective bruxism is determined by asking the participants if they believe they perform bruxism activity, and if so in what frequency. A distinction between awake bruxism and sleep bruxism will be made. [32]

Objective bruxism is determined based on several intra-oral aspects, i.e., the presence or absence of a bruxoposition (the occlusal contact position to which one grinds and in which one clenches), impressions of the soft tissues (cheeks, tongue, and/or lips), the suspected nature – being mechanical, chemical, or both – of existing tooth wear, and quantification of existing tooth wear. The quantification of tooth wear is recorded using the screening module of the comprehensive Tooth Wear Evaluation System (TWES), [33] resulting in a score of 0 to 4, with 0 being "no (visible) wear" and 4 being "loss of clinical crown height >2/3", for occlusal/incisal wear for each sextant, [34] and a score of 0 to 2, with 0 being "no wear" and 2 being "wear with dentine exposed", for wear on the palatinal surfaces of the second sextant.

Findings will be used to determine a bruxism diagnosis according to the diagnostic grading system proposed by Lobbezoo et. al., [32,35] where 'possible' sleep or awake bruxism will be diagnosed based on self-report, and 'probable' sleep or awake bruxism will be diagnosed based on the combination of self-report and clinical findings.

Oral microbiome

During the baseline visit, samples for oral microbiome analyses will be collected. The oral microbiome has been reported to significantly differ between different oral niches, [36] and therefore a variety of samples will be collected. Subgingival dental plaque will be collected from the first molar of the fourth quadrant (when needed, the other molars and premolars will be used in a standardized order), using a sterile universal curette. Participants will be asked to chew paraffin to enable the collection of a stimulated saliva sample. A sample of the tongue coating will be collected by swiping a microbrush on the dorsum of the tongue.

The microbiome of these samples will be assessed to determine a possible presence and possible role of a dysbiosis in the development of RA. Based on the 16S rRNA, species will be grouped in Operational Taxonomic Units (OTU's), after which samples can be grouped in clusters based on similarities using Neighborhood Co-regularized Spectral Clustering (NCSC), an approach used in previous microbial ecological studies. [37,38] These clusters will be used as the microbiological outcome value for further analyses.

In addition to the collection of samples, the fluorescence of dental plaque will be studied. [39] Participants will be asked to refrain from oral hygiene (i.e., brushing the teeth, using interdental devices, mouth wash, and chewing gum) 24 hours prior to their baseline visit. During the baseline visit, a total of six photographs of the dentition will be taken – three fluorescence photographs, and three corresponding white light photographs. To be able to explore a possible influence of nutrition on plaque fluorescence, all participants will be asked to keep a food diary during the three days prior to their baseline visit.

Intra-oral immuno-biochemical characteristics

Gingival Crevicular Fluid (GCF), containing cells related to the immune system and therefore portraying immune-biochemical characteristics, will be collected at baseline. [40] GCF will be collected at four sites – the mesial sides of the upper canines and the distal sides of the upper central incisors – by means of a sterile paper strip at the entrance of the crevice. The paper strip will be held in place during 30 seconds and a new paper strip will be used for each site.

Quantification of the absorbed GCF will be determined immediately after removing the paper strip, by using a same-day calibrated Periotron 8000 (Oraflow Inc., New York, NY, USA). Because local conditions have been described to influence outcome values of the Periotron 8000, [41] both temperature and humidity will be recorded during calibration and GCF collection to be able to detect possible substantial differences between locations or over time.

Oral health related quality of life (OHRQoL)

Prior to the baseline visit, participants will be asked to fill in the shortened Oral Health Impact Profile questionnaire (OHIP-14), derived from the original 49-item OHIP, a tool for assessing the social impacts of oral disorders. [42] With the OHIP-14, the frequency of a variety of possible impacts (14 questions) during the past month is scored on a 5-point ordinal scale, ranging from 0 ("never") to 4 ("very often"), resulting in a total score ranging from 0 to 56. A Dutch translation of the OHIP-49 has been developed and validated, [43] and the shorted OHIP-14 has been reported adequate for replacing the original 49-item OHIP. [44]

Self-reported disease status
At Reade, patients' self-reported physical function, pain, and global estimate of disease status are periodically measured by the RAPID-3 questionnaire – a Routine Assessment of Patient Index Data described to be valuable for measuring disease status. [45] If recent RAPID-3 data for a participant is available at Reade, this data will be used for the current study. If no recent data is available, participants will be asked to fill in the RAPID-3 questionnaire prior to their baseline visit. This also applies to participants in the control group.

The first section of the RAPID-3, physical function, is scored for several activities on a scale from 0 ("without any difficulty") to 3 ("unable to do"), converted to a total score ranging from 0 to 10. The second (pain) and third (global estimate of disease status) sections of the questionnaire are scored on a scale from 0 ("no pain"/ "very well" respectively) to 10 ("pain as bad as it could be"/ "very poorly" respectively). The RAPID-3 results in an overall score ranging from 0 to 30.

Xerostomia
To measure symptoms of xerostomia, participants will be asked to fill in the Xerostomia Inventory questionnaire [46] prior to the baseline visit. The questionnaire contains 11 questions about the frequency in which someone had to act on, or had trouble functioning because of, the adverse consequences of xerostomia during the past four weeks, scored on a scale from 1 ("never") to 5 ("very often").

Statistical analysis
Descriptive statistics
Characteristics of the study cohort will be reported. For continuous variables, the mean, median, standard deviation, minimum and maximum values (or the range) will be reported, together with a percentage of frequency values.

Analyses of study parameters
The differences between groups in the presence of a defined TMD diagnosis will be analyzed using the Chi-square test. Differences in outcomes of the several separate DC/TMD tests – not necessarily resulting in a defined diagnosis – will be analyzed with the Friedman test (one-way repeated measures analysis of variance by ranks). For the longitudinal outcomes as well as some of the secondary outcomes, a Generalized Estimation Equation analysis will be used. A two-sided alpha level of 0.05 will be used for all statistical analysis.

An interim analysis will be performed when approximately 20 subjects in each group have been included. Cumulative frequency analysis will be used to predict future outcomes, and to determine if including the planned amount of subjects will add value to the existing results. By performing an interim analysis, possible unnecessary burden for the (future) participants can be prevented.

Ethics approval and consent to participate
The study protocol has been approved by the accredited Medical Ethical Committee of the Slotervaart Hospital and Reade (*METc Slotervaartziekenhuis en Reade*, U/17.056/P1719) according to the Declaration of Helsinki (2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The study has been included in the general assessment and registration form (ABR form, NL61521.048.17) and the Dutch National Trial Register (NTR, NL6198). Written informed consent is obtained from all study participants.

Discussion

This research proposal for a prospective cohort study is intended to investigate the orofacial tissues – including the TMJ, oral health, and the oral microbiome – in patients with early RA and those at increased risk of developing RA. Although the strengths of this study protocol lie in extensive collection of research data at baseline and the longitudinal design, some limitations should also be mentioned.

Insufficient data is available to accurately calculate the group sizes that are required to explore TMD prevalence in the various groups. This makes the study design challenging. However, by using comprehensive tests to examine the masticatory system, distinguishing both arthrogenous and myogenous components, and by focusing on two very specific patient groups, we believe this study could provide a valuable addition to the limited data currently available. If our results indicate a difference in prevalence between groups 1 and 2 and the control group, a larger validation study could be planned to further explore this topic. Although Reade is seen as the RA expertise center and the referral clinic for a large area in the western Netherlands, the number of people at increased risk of RA who are being referred is still limited. A future multi-center study, possibly international, might therefore be needed to include a larger number of subjects as to increase the generalizability.

With regard to groups 1 and 2, our dependence on the patient intakes at Reade will not enable us to achieve one-to-one matching on age and sex for these two groups. However, we anticipate that subjects in group 1 and 2 will not

differ significantly in age or sex distribution, as the increased risk of developing RA is a possible precursor of early RA. To approach accurate matching of the control group, recruitment of this group will start with a few months delay, aiming for a control group with an average age and sex distribution that does not statistically differ from the two other groups.

It is known that medication can significantly influence oral health and the oral microbiome, [29,47] and as the effects of anti-rheumatic agents have been described specifically, [48] patients' medication is an important factor that may create a wide variety within group 1. As we expect that basing subgroups on medication use will greatly fragment group 1, we will analyze the results for this group as a whole, considering medication as a potential confounding factor.

As for the determination of a TMD diagnosis, there is a minor limitation. If after clinical examination a diagnosis is still uncertain, the DC/TMD describes 'imaging' by MRI or CT as the final step for confirming certain diagnoses (disc displacement and degenerative joint disease). [49] However, imaging is not feasible within the scope of this study. On the other hand, it has also been suggested that imaging should be considered only if the information it provides would influence patient care. [50] Therefore, since this study will not indicate any treatment, there is no basis for imaging. A similar limitation applies to the 'definite' diagnosis of bruxism: as no polysomnographic recording or electromyographic recording will be performed, the diagnosis will be limited to 'possible' or 'probable' sleep or awake bruxism according to the diagnostic grading system for bruxism. [32]

Despite these limitations, we argue that the outcomes of this study will provide new and important insight into a wide variety of factors regarding the interaction between RA and the orofacial tissues. It may thus indicate a direction for further research in this area.

References

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376:1094-1108.
2. Arora S, Rafiq A, Jolly M. Management of rheumatoid arthritis: Review of current guidelines. J Arthrosc Joint Surg. 2016;3:45-50.
3. Nell VP, Machold KP, Eberl G, Stamm TA, Uffmann M, Smolen JS. Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. Rheumatology (Oxford). 2004;43:906-914.
4. van Dongen H, van Aken J, Lard LR, Visser K, Ronday HK, Hulsmans HM, et al. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum. 2007;56:1424-1432.
5. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nat Rev Immunol. 2017;17:60-75.
6. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004;50:380-386.
7. van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis. 2013;72:1920-1926.
8. Rakieh C, Nam JL, Hunt L, Hensor EM, Das S, Bissell LA, et al. Predicting the development of clinical arthritis in anti-CCP positive individuals with non-specific musculoskeletal symptoms: a prospective observational cohort study. Ann Rheum Dis. 2015;74:1659-1666.
9. Moen K, Bertelsen LT, Hellem S, Jonsson R, Brun JG. Salivary gland and temporomandibular joint involvement in rheumatoid arthritis: relation to disease activity. Oral Dis. 2005;11:27-34.
10. Sidebottom AJ, Salha R. Management of the temporomandibular joint in rheumatoid disorders. Br J Oral Maxillofac Surg. 2013;51:191-198.
11. Yildizer Keris E, Yaman SD, Demirag MD, Haznedaroglu S. Temporomandibular joint findings in patients with rheumatoid arthritis, ankylosing spondylitis, and primary Sjogren's syndrome. J Investig Clin Dent. 2016;8:e12255.
12. Bono AE, Learreta JA, Rodriguez G, Marcos JC. Stomatognathic system involvement in rheumatoid arthritis patients. Cranio. 2014;32:31-37.
13. Wolfe F, Katz RS, Michaud K. Jaw pain: its prevalence and meaning in patients with rheumatoid arthritis, osteoarthritis, and fibromyalgia. J Rheumatol. 2005;32:2421-2428.
14. Lin Y-C, Hsu M-L, Yang J-S, Liang T-H, Chou S-L, Lin H-Y. Temporomandibular Joint Disorders in Patients with Rheumatoid Arthritis. Journal of the Chinese Medical Association. 2007;70:527-534.
15. Chin Jen Sem JP, van der Leeden M, Visscher CM, Britsemmer K, Turk SA, Dekker J, et al. Prevalence, Course, and Associated Factors of Pain in the Temporomandibular Joint in Early Rheumatoid Arthritis: Results of a Longitudinal Cohort Study. J Oral Facial Pain Headache. 2017;31:233-239.
16. Pischon N, Pischon T, Kroger J, Gulmez E, Kleber BM, Bernimoulin JP, et al. Association

among rheumatoid arthritis, oral hygiene, and periodontitis. J Periodontol. 2008;79:979-986.
17. Dissick A, Redman RS, Jones M, Rangan BV, Reimold A, Griffiths GR, et al. Association of periodontitis with rheumatoid arthritis: a pilot study. J Periodontol. 2010;81:223-230.
18. Arkema EV, Karlson EW, Costenbader KH. A prospective study of periodontal disease and risk of rheumatoid arthritis. J Rheumatol. 2010;37:1800-1804.
19. Demmer RT, Molitor JA, Jacobs DR, Jr., Michalowicz BS. Periodontal disease, tooth loss and incident rheumatoid arthritis: results from the First National Health and Nutrition Examination Survey and its epidemiological follow-up study. J Clin Periodontol. 2011;38:998-1006.
20. Eriksson K, Nise L, Kats A, Luttropp E, Catrina AI, Askling J, et al. Prevalence of Periodontitis in Patients with Established Rheumatoid Arthritis: A Swedish Population Based Case-Control Study. PLoS One. 2016;11:e0155956.
21. Mikuls TR, Payne JB, Yu F, Thiele GM, Reynolds RJ, Cannon GW, et al. Periodontitis and Porphyromonas gingivalis in patients with rheumatoid arthritis. Arthritis Rheumatol. 2014;66:1090-1100.
22. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, et al. The oral microbiome - an update for oral healthcare professionals. Br Dent J. 2016;221:657-666.
23. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62:2569-2581.
24. De Kanter RJ, Truin GJ, Burgersdijk RC, Van 't Hof MA, Battistuzzi PG, Kalsbeek H, et al. Prevalence in the Dutch adult population and a meta-analysis of signs and symptoms of temporomandibular disorder. J Dent Res. 1993;72:1509-1518.
25. Visscher CM, Ligthart L, Schuller AA, Lobbezoo F, de Jongh A, van Houtem CM, et al. Comorbid disorders and sociodemographic variables in temporomandibular pain in the general Dutch population. J Oral Facial Pain Headache. 2015;29:51-59.
26. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. J Oral Facial Pain Headache. 2014;28:6-27.
27. Ohrbach R, Gonzalez YM, List T, Michelotti A, Schiffman E. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD): Clinical Examination Protocol. Version 06 January 2014. Available from: www.rdc-tmdinternational.org.
28. Visscher CM, Naeije M, De Laat A, Michelotti A, Nilner M, Craane B, et al. Diagnostic accuracy of temporomandibular disorder pain tests: a multicenter study. J Orofac Pain. 2009;23:108-114.
29. Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MP, Rashid MU, et al. Same Exposure but Two Radically Different Responses to Antibiotics: Resilience of the Salivary Microbiome versus Long-Term Microbial Shifts in Feces. MBio. 2015;6:e01693-01615.
30. Silness J, Loe H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. Acta Odontol Scand. 1964;22:121-135.
31. Gruebbel AO. A Measurement of Dental Caries Prevalence and Treatment Service for Deciduous Teeth. J Dent Res. 1944:163-168.
32. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, et al. Bruxism defined and graded: an international consensus. J Oral Rehabil. 2013;40:2-4.
33. Wetselaar P, Lobbezoo F. The tooth wear evaluation system: a modular clinical guideline for the diagnosis and management planning of worn dentitions. J Oral Rehabil. 2016;43:69-80.
34. Lobbezoo F, Naeije M. A reliability study of clinical tooth wear measurements. J Prosthet Dent. 2001;86:597-602.
35. Lobbezoo F, Ahlberg J, Raphael KG, Wetselaar P, Glaros AG, Kato T, et al. International consensus on the assessment of bruxism: Report of a work in progress. J Oral Rehabil. 2018;45:837-844.
36. Xu X, He J, Xue J, Wang Y, Li K, Zhang K, et al. Oral cavity contains distinct niches with dynamic microbial communities. Environ Microbiol. 2015;17:699-710.
37. Zaura E, Brandt BW, Prodan A, Teixeira de Mattos MJ, Imangaliyev S, Kool J, et al. On the ecosystemic network of saliva in healthy young adults. ISME J. 2017;11:1218-1231.
38. Biesbroek G, Tsivtsivadze E, Sanders EA, Montijn R, Veenhoven RH, Keijser BJ, et al. Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. Am J Respir Crit Care Med. 2014;190:1283-1292.
39. Volgenant CM, Hoogenkamp MA, Krom BP, Janus MM, Ten Cate JM, de Soet JJ, et al. Red and Green Fluorescence from Oral Biofilms. PLoS One. 2016;11:e0168428.
40. Griffiths GS. Formation, collection and significance of gingival crevice fluid. Periodontol 2000. 2003;31:32-42.
41. Tozum TF, Hatipoglu H, Yamalik N, Gursel M, Alptekin NO, Ataoglu T, et al. Critical steps in electronic volume quantification of gingival crevicular fluid: the potential impact of evaporation, fluid retention, local conditions and repeated measurements. J Periodontal Res. 2004;39:344-357.
42. Slade GD, Spencer AJ. Development and evaluation of the Oral Health Impact Profile. Community Dent Health. 1994;11:3-11.
43. van der Meulen MJ, John MT, Naeije M, Lobbezoo F. The Dutch version of the Oral Health Impact Profile (OHIP-NL): Translation, reliability and construct validity. BMC Oral Health. 2008;8:11.
44. van der Meulen MJ, John MT, Naeije M, Lobbezoo F. Developing abbreviated OHIP versions for use with TMD patients. J Oral Rehabil. 2012;39:18-27.
45. Pincus T, Bergman MJ, Yazici Y, Hines P, Raghupathi K, Maclean R. An index of only patient-reported outcome measures, routine assessment of patient index data 3 (RAPID3), in two abatacept clinical trials: similar results to disease activity score (DAS28) and other RAPID indices that include physician-reported measures. Rheumatology (Oxford). 2008;47:345-349.
46. Thomson WM, Chalmers JM, Spencer AJ, Williams SM. The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health. 1999;16:12-17.
47. Ciancio SG. Medications' impact on oral health. J Am Dent Assoc. 2004;135:1440-1448; quiz 1468-1449.
48. Han JY, Reynolds MA. Effect of anti-rheumatic agents on periodontal parameters and

biomarkers of inflammation: a systematic review and meta-analysis. J Periodontal Implant Sci. 2012;42:3-12.
49. Ohrbach R (editor). Diagnostic Criteria for Temporomandibular Disorders: Assessment Instruments. Version 15 May 2016. Available from: www.rdc-tmdinternational.org.
50. Peck CC, Goulet JP, Lobbezoo F, Schiffman EL, Alstergren P, Anderson GC, et al. Expanding the taxonomy of the diagnostic criteria for temporomandibular disorders. J Oral Rehabil. 2014;41:2-23.

Temporomandibular joint disorders: prevalence

Temporomandibular disorders in patients with early rheumatoid arthritis and at-risk individuals in the Dutch population: a cross-sectional study

Johanna M. Kroese, Catherine M.C. Volgenant, Wim Crielaard, Bruno G. Loos, Dirkjan van Schaardenburg, Corine M. Visscher, Frank Lobbezoo

Published in: RMD Open. 2021;7(1):e001485.
PMID: 33397683.

Chapter 03

ABSTRACT

Objective: To evaluate the prevalence of temporomandibular disorders (TMD) in patients with early rheumatoid arthritis (ERA) and individuals at risk of RA.

Methods: 150 participants were recruited in three groups (50 per group): (1) patients with ERA (2010 EULAR criteria), (2) at-risk individuals, and (3) healthy controls. All participants were tested for seropositivity of rheumatoid factor and anticitrullinated protein antibodies. A possible TMD diagnosis was determined according to the standardized and validated diagnostic criteria for TMD (DC/TMD) in five categories: myalgia, arthralgia, articular disc displacement, degenerative joint disease, and headache attributed to TMD. Results were tested for the prevalence of TMD (all categories combined) and TMD pain (myalgia and/or arthralgia). To investigate a possible role for bruxism, a probable sleep and/or awake bruxism diagnosis was determined based on self-report and several clinical features.

Results: The prevalence of any TMD diagnosis did not differ between the three groups. However, at-risk individuals more often had a TMD-pain diagnosis than healthy controls (p=0.046). No such difference was found between the ERA-group and the control group. However, within the ERA-group, seronegative patients had a TMD-pain diagnosis more often than seropositive patients (4/12 (33%) versus 3/38 (8%), p=0.048). Participants with a TMD-pain diagnosis were more often diagnosed with probable sleep bruxism than those without a TMD-pain diagnosis.

Conclusion: The prevalence of TMD pain is increased in individuals at risk of RA and seronegative ERA patients, and is associated with bruxism signs and symptoms. These results suggest that health professionals should be alert to TMD pain in these groups.

Background

Rheumatoid Arthritis (RA) is an auto-immune disease that causes inflammation of the synovial joints, eventually resulting in destruction of cartilage and bone. [1] Most RA patients are affected by the so-called seropositive form of RA, defined by the presence of specific antibodies: IgM rheumatoid factor (IgM-RF) and antibodies against citrullinated proteins (ACPA). [2] According to the 2010 European League Against Rheumatism (EULAR) criteria, diagnosis of RA depends on a scoring system including several factors, e.g., IgM-RF and/or ACPA positivity and symptom duration, while a definite synovitis in at least one joint is required. [3] In the Dutch population, approximately 17.000 new patients (incidence of 0,1% per year) get diagnosed with RA each year. [4]

RA can also affect the temporomandibular joint (TMJ). The prevalence of TMJ involvement in patients with RA has been reported to range between 19% and 86%. [5-7] As previous studies describe a wide variation in diagnostic criteria, assessment methods, and RA disease duration, it is difficult to determine the overall prevalence and possible variations during the course of the disease.

Data on TMJ involvement in early RA (ERA) is limited; solely based on palpation, Chin Jen Sem et al. found an 11% prevalence of TMJ pain in patients with newly diagnosed RA. [8] As several characteristics of RA can be present before the clinical outbreak of arthritis, e.g., arthralgia and increased serum levels of IgM-RF and/or ACPA, individuals with an elevated risk of RA can be identified. [9] Currently, no information is available on the prevalence of TMJ involvement in people at risk of RA. Research on both patients with ERA and individuals at risk of RA would therefore be a valuable addition to the available literature, and could also be of value for the collaboration between rheumatologists and dentists around RA onset in the TMJ.

To fully explore possible TMJ involvement in ERA patients and at-risk individuals, it would be preferable to consider all disorders of the TMJ. These comprise pain and dysfunction of the TMJ and the masticatory muscles as well as TMJ sounds (i.e., clicking and/or crepitus) during function. To systematically examine the masticatory system and to arrive at a temporomandibular disorder (TMD) diagnosis, the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) have been composed and validated by an international consortium. [10] The DC/TMD uses standardized tests, taking into account both arthrogenous and myogenous aspects. Bruxism – defined as a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible [11,12] – is reported to play a role in the development of TMD. [13] It is therefore a relevant factor to consider when analysing TMD in any population.

Insight into TMD prevalence around RA onset would provide information on whether alertness to a possible TMD during this timeframe is needed. Our aim is therefore to evaluate the prevalence of TMD according to the DC/TMD, and a possible role for bruxism, in patients with ERA and in individuals with an increased risk of RA compared to a healthy control group. We hypothesize that the prevalence of TMD in both ERA and at-risk individuals is higher than in healthy controls.

Methods

Study design and ethical approval

This study is part of a larger parent study. A full description of the study protocol has been published. [14] The study protocol has been approved by the Medical Ethical Committee of the Slotervaart Hospital and Reade (METc Slotervaartziekenhuis en Reade, U/17.056/P1719), and has been registered in the Dutch National Trial Register (NTR, NTR6362).

Participants and recruitment

Three groups of participants were recruited: (1) patients with ERA, (2) persons with an increased risk of RA, and (3) a control group without auto-immune conditions. Subjects were eligible for inclusion if they were aged 18 years or older, had a minimum of 12 natural teeth, and were willing and able to give written informed consent. Groups 1 and 2 were recruited at Reade, a rheumatology clinic in Amsterdam, The Netherlands. For group 1, patients were diagnosed with RA and fulfilled the 2010 EULAR RA criteria [3] within the last year. From January 2018, newly diagnosed patients were approached and informed about this study by a nurse of Reade. For group 2, from November 2017, new participants in the Reade at-risk cohort, [15-17] and inclusions up to 6 months retrospectively, were approached by a physician. Participants in this cohort have the combination of inflammatory-type arthralgia and increased serum levels of IgM-RF and/or ACPA. After oral consent to the nurse or physician of Reade, potential participants for groups 1 and 2 were contacted, thoroughly informed about this study, and eventually recruited by a dentist (JMK).

Participants for the control group (group 3) were recruited from among the regular dental patient population at the Academic Centre for Dentistry Amsterdam (ACTA), and from among a group of people who expressed interest in participating in research at ACTA. Recruitment was done by a dentist (JMK), either directly or after initial approach by a dental student of ACTA. Control subjects were matched to groups 1 and 2 for sex and age.

Potential participants were approached until the targeted amount of 50 participants in each group was reached, which occurred in March 2019 for group 2 and in July 2019 for groups 1 and 3. All research visits took place at Reade and all clinical examinations were performed by one trained dentist (JMK). Because all recruitment and scheduling of research appointments was performed by the same dentist, blinding of the examiner was not feasible.

Outcome variables

General health status

Prior to the research visit, all subjects completed a medical questionnaire to identify possible confounders, such as comorbid disorders and medication use. During the research visit, additional questions were asked about recent use of analgesics because of the potential masking effect on TMD pain during the clinical examination.

Venous blood was collected to determine serum levels of IgM-RF and ACPA; individuals with IgM-RF levels of >5.0 kU/l and/or ACPA levels of >10.0 kU/l were considered seropositive; otherwise, subjects were considered seronegative.

For ERA patients, the DAS28 score [18] was determined by a trained registered nurse of Reade. A DAS28 score of <2.6 is associated with being in remission according to the American Rheumatism Association (ARA) criteria. [19] The ERA patients were also asked to complete the RAPID-3 questionnaire, resulting in a score ranging from 0 to 10, representing the subjective disease status. [20]

Temporomandibular joint disorders

The presence of a TMD was classified according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). [10] Five diagnostic categories were recognized: (1) myalgia, (2) arthralgia, (3) disc displacement, (4) degenerative joint disease, and (5) headache attributed to TMD. Prior to the research visit, all participants filled out the DC/TMD symptom questionnaire with 14 questions on pain in the joint area, headache, joint sounds, and joint locking (see reference for URL). [21,22]

The clinical examination was performed according to the DC/TMD Clinical Examination Protocol. [23] To determine a possible TMD-pain diagnosis (myalgia or arthralgia), the clinical examination included the measurement of maximum mandibular movements (opening, protrusion, and laterotrusion to both sides), and the registration of possible pain in the TMJs and/or surrounding

muscles during these movements. Pain on palpation was recorded on both the masseter muscles and the temporal muscles (nine points on each muscle), and on the lateral pole and around the lateral pole of both TMJ condyles. To be diagnosed with myalgia or arthralgia, a participant had to report familiar pain during one of the clinical tests in the muscle or joint area, respectively, combined with reported orofacial pain that is influenced by jaw activity during the past 30 days on the questionnaire.

Non-painful symptoms, e.g., unpleasantness, tension, and soreness, were also recorded during the clinical examination. When a participant reported pain on the questionnaire and recognized the location of unpleasant non-painful symptoms, but not pain, during the clinical examination, these participants were categorized as having a DC/TMD diagnosis. This decision was made based on the current insight that both pain and non-painful symptoms are associated with TMD pain. [24]

A possible disc displacement or degenerative joint disease diagnosis was based on the presence of clicking sounds or crepitation, respectively. Participants were asked to perform each of the following jaw movements three times: open and close, protrusion, and laterotrusion to both sides. To be diagnosed with a disc-displacement or degenerative joint disease, respectively, the click or crepitation should be heard by, or be palpable to the examiner during the examination, combined with reported sounds by the participant on the questionnaire or during the examination.

To be diagnosed with a headache attributed to TMD, a participant had to report a familiar headache induced by jaw movement or palpation during the clinical examination, combined with reported headache in the temporal region that is influenced by jaw activity on the questionnaire, and a TMD-pain diagnosis in the category myalgia and/or arthralgia.

The dentist performing the clinical research (JMK) was trained in performing the described examinations by a specialist in TMD who was calibrated by the International Network for Orofacial Pain and Related Disorders Methodology (INfORM) consortium. [25]

Bruxism

To study a possible role of bruxism, both subjective and objective bruxism activity were measured. These findings were used to determine a bruxism diagnosis according to the diagnostic grading system proposed by Lobbezoo et al. [11,12] According to this grading system, 'possible' bruxism is diagnosed based on self-report, and thus patients were asked about their assumption of performing bruxism activity while awake and/or during sleep using the questions from the DC/TMD oral behaviours questionnaire. [21] Participants were diagnosed with possible bruxism if they reported grinding and/or clenching with a frequency of at least '1-3 nights a month' (sleep bruxism) or 'sometimes' (awake bruxism).

'Probable' bruxism is diagnosed based on the combination of self-report and clinical findings, i.e., the presence of at least one of the following: a bruxoposition (i.e., an obvious and reproducible contact position of the teeth of the upper and lower jaw, indicating grinding towards, and/or clenching in this contact position), impressions of the teeth in the soft tissues (cheeks, tongue, and/or lips), and tooth wear of mechanical nature with a minimum grade 2 according to the Tooth Wear Evaluation System (TWES). [26]

Statistical analysis

Descriptive statistics were used to describe the characteristics of the study cohort. For continuous variables, the independent samples t-test was used when comparing the means of two groups, and the one-way ANOVA was used when comparing the means of more than two groups. Differences between groups on binary variables were tested with a Chi-square test, or Fisher's exact test when appropriate. A two-sided alpha level of 0.05 was used for all statistical analyses.

For the primary outcome, i.e., prevalence of TMD and TMD-pain diagnoses, the three study groups were compared. Additionally, within the ERA group, seropositive and seronegative patients were compared. Secondary, to test for a possible relation between the prevalence of TMD (pain) and other variables, e.g., use of analgesic medication during the past 24 hours and mandibular movement capacity, participants with and without a TMD(-pain) diagnosis within the total study population were compared. For analyses including bruxism as a variable, the probable bruxism diagnoses were used.

Results

Characteristics of the study sample

From November 2017 until July 2019, 150 participants were included, 50 per group. Table 1 displays the characteristics of the study sample. The ERA patients were included in the study after being diagnosed with RA for an average of 3.1±1.7 months. The time since RA diagnosis was longer for seronegative patients than for seropositive patients (3.8±2.2 vs 2.9±1.4 months, p=0.011). The majority (88%) of ERA patients was treated with methotrexate, mostly in combination with prednisone, according to the Dutch guideline on drug treatment of RA [27] (table 1).

Table 1. Characteristics of the study sample

	ERA group (n=50)	At-risk group (n=50)	Control group (n=50)	*p*
Age, years [mean (SD)]	52.1 (13.2)	51.4 (10.3)	51.2 (11.0)	0.923[a]
Gender, female [n (%)]	39 (78%)	38 (76%)	38 (76%)	0.963[b]
IgM-RF positive [n (%)]	37 (74%)	46 (92%)	0 (0%)	-[c]
ACPA positive [n (%)]	31 (62%)	24 (48%)	0 (0%)	-[c]
DAS28 [mean (SD)]	2.61 (1.17)	-	-	-
RAPID-3 [median (IQR)]	3.09 (1.07-4.47)	-	-	-
Analgesic medication <24h [n (%)]	12 (24%)	16 (32%)	9 (18%)	0.265[b]
Pharmacological treatment for RA				
Methotrexate [n (%)]	44 (88%)	-	-	-
Other [n (%)]	4 (8%)	-	-	-
No pharmacological treatment [n (%)]	2 (4%)	-	-	-
Prednisone [n (%)]	39 (78%)	-	-	-

ERA = early rheumatoid arthritis, n = number of observations, IgM-RF = IgM rheumatoid factor, ACPA = anti-citrullinated protein antibodies, DAS = disease activity score.

[a] One-way ANOVA, F = 0.080

[b] Chi-square test, Chi-square = 0.075

[c] A difference in IgM-RF or ACPA positivity was not tested between the groups, because seropositivity was an inclusion criterion for the at-risk group and an exclusion criterion for the control group, and a difference is thus obvious.

Temporomandibular disorders

No significant differences were found for the prevalence of TMD diagnoses between the three study groups (table 2). Because a temporomandibular disc displacement is a very common condition in the general population that usually requires no treatment, [28] results are also reported for numbers of participants with a TMD-pain diagnosis (either myalgia, arthralgia, or both), and for having a degenerative joint disease diagnosis (table 2). A significantly higher number of participants in the at-risk group had a TMD-pain diagnosis compared to the control group (p=0.046). No such difference was found when comparing the ERA group to the control group. However, within the ERA-group, seronegative patients had a TMD-pain diagnosis significantly more often than seropositive patients (4/12 (33%) versus 3/38 (8%), p=0.048).

Table 2. Prevalence of Temporomandibular Disorders (TMD), TMD pain, degenerative joint disease (DJD), and mandibular movement capacity in the study sample

	ERA group (n=50)	At-risk group (n=50)	Control group (n=50)	Test statistics	*p*
Diagnosis according to DC/TMD					
TMD diagnosis[1] [n (%)]	20 (40%)	19 (38%)	14 (28%)	χ2= 1.604[3], 1.131[4]	0.205[3a], 0.288[4a]
TMD-pain diagnosis[2] [n (%)]	7 (14%)	8 (16%)	2 (4%)	χ2= 4.000[4]	0.16[3b], 0.046[4a]
DJD diagnosis [n (%)]	6 (12%)	2 (4%)	2 (4%)		0.269[3b], 1.0[4b]
Bruxism					
Probable sleep bruxism [n (%)]	9 (18%)	14 (28%)	11 (22%)		0.485[a]
Mandibular movement capacity					
Maximum mouth opening, mm [mean (SD)]	49.1±7.2	51.4±5.2	50.7±6.3	F = 1.815	0.167[c]
Maximum protrusion, mm [mean (SD)]	8.5±2.1	7.8±2.5	8.2±2.8	F = 0.836	0.436[c]
Maximum laterotrusion, mm [mean (SD)]	10.6±2.3	10.7±2.4	9.9±3.0	F = 1.332	0.267[c]

n = number of observations, TMD = temporomandibular disorders, DJD = degenerative joint disease, ERA = early rheumatoid arthritis

[1] myalgia, arthralgia, disc displacement, DJD and/or headache attributed to TMD, [2] myalgia and/or arthralgia

[3] ERA-group versus control group, [4] At-risk group versus control group

[a] Chi-square test (χ2), [b] Fisher's Exact, [c] One-way ANOVA (F)

Sixteen out of 17 participants with a TMD-pain diagnosis reported on the duration of their pain complaints, with a median (IQR) of 15 (4-138) months. For six out of eight (75%) participants with a myalgia, the diagnosis was bilateral. For arthralgia, six out of twelve participants (50%) had a bilateral diagnosis, and another three participants (25%) had unfamiliar pain or non-painful symptoms on the contralateral side. A total of 37 participants reported to have used analgesic medication, mostly paracetamol, during the past 24 hours. There was no difference in analgesic medication use between participants with or without a TMD-pain diagnosis (p=0.765).

No difference was found between the groups in mandibular movement capacity, i.e., maximum mouth opening, protrusion, and laterotrusion (table 2). For laterotrusion, an average of the laterotrusion to the left and to the right was used for analysis, because there was no significant difference in laterotrusion between the painful and non-painful side (p=0.933) in participants with unilateral TMD pain. When comparing participants with and without a TMD-pain diagnosis in the total study population, also no difference was found in mandibular movement capacity (opening p=0.906, protrusion p=0.788, laterotrusion p=0.333).

Bruxism

The number of participants reporting on awake bruxism only – five in total – was too small for statistical analysis, and thus only results for sleep bruxism were further analysed. Thirty-five participants reported possible sleep bruxism activity, of which 34 participants were diagnosed with probable sleep bruxism – nine in the ERA group, 14 in the at-risk group, and 11 in the control group – with no significant difference between the groups (p=0.485).

No difference was found in probable sleep bruxism diagnosis between participants with and without any TMD diagnosis (15/53 (28%) versus 19/97 (20%), p=0.223). However, participants with a TMD-pain diagnosis had a probable sleep bruxism diagnosis more often than participants without a TMD-pain diagnosis (8/17 (47%) versus 26/133 (20%), p=0.011).

Relation to disease activity in the ERA-group

Within the ERA-group, no difference in the prevalence of TMD (p=0.355) or TMD-pain (p=0.697) was found between patients with a DAS28 score <2.6 and patients with a DAS28 score ≥2.6. However, the RAPID-3 score was significantly higher for patients with a TMD diagnosis compared to patients without a TMD diagnosis (3.76±1.97 versus 2.46±2.06, p=0.031). This difference was not found between patients with or without a TMD-pain diagnosis (3.72±2.40 versus 2.86±2.06, p=0.324).

The RAPID-3 score did not differ between seropositive and seronegative ERA patients (3.0±1.8 versus 3.0±2.2, p=0.982). However, when comparing the DAS28 score of both groups, seronegative patients were more often categorized as being in remission compared to seropositive patients (10/12 (83%) versus 14/38 (37%), p=0.005).

Pain and non-painful symptoms without a DC/TMD diagnosis

In addition to the official DC/TMD classifications, figures 1A-B show the prevalence of unfamiliar pain and non-painful symptoms during the clinical examination for the categories myalgia and arthralgia, respectively.

In addition to the 17 participants with a TMD-pain diagnosis, a total of eight participants – five in the ERA-group, two in the at-risk group, and one in the control group – reported pain influenced by jaw function on the questionnaire, but did not confirm familiar pain or non-painful symptoms during the clinical examination. The positive predictive value of the DC/TMD questionnaire in the present study population is thus (17/(17+8)=) 68%.

In the ERA group, two patients reported familiar pain in the TMJ during the clinical examination and pain in the questionnaire, but not during the past 30 days, and thus did not receive a DC/TMD arthralgia diagnosis. Two other ERA patients reported previous pain in the TMJ, which resolved since the start of pharmacological treatment for RA. One ERA patient with an arthralgia diagnosis reported to have less pain after taking daily doses of prednisone. Out of these five patients, four were seropositive ERA patients.

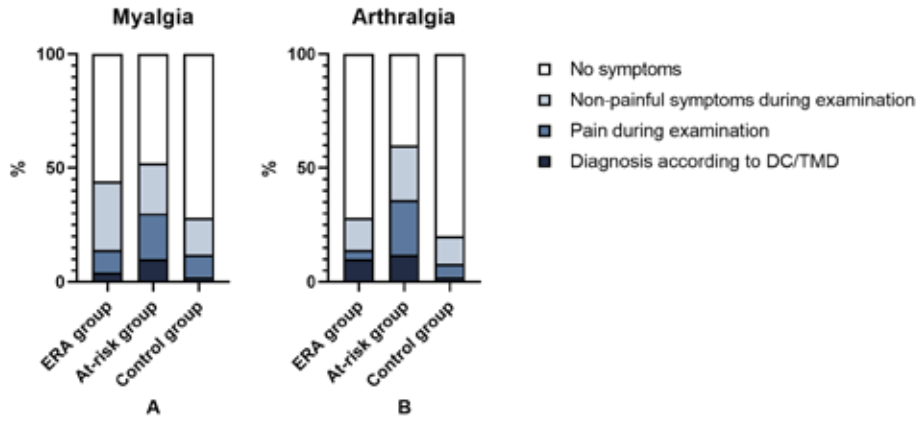


Figure 1. Percentages of participants with a diagnosis according to the diagnostic criteria for temporomandibular dysfunction (DC/TMD), unfamiliar pain during examination, non-painful symptoms during examination, and no symptoms. A, results for myalgia; B, results for arthralgia.

Discussion

The prevalence of temporomandibular disorders (TMD) according to the diagnostic criteria for TMD (DC/TMD) was investigated cross-sectionally in patients with early rheumatoid arthritis (ERA), individuals at risk of developing RA, and an age and sex matched healthy control group. The three groups did not differ when comparing them based on the prevalence of TMD, i.e., myalgia, arthralgia, disc displacement, degenerative joint disease, and headache attributed to TMD combined. However, when considering TMD-pain diagnoses only – myalgia and/or arthralgia – participants in the at-risk group had a higher prevalence of TMD pain than those in the control group. These results suggest that medical professionals should be alert for TMD-pain disorders in individuals at risk of RA, in order to identify individuals that might benefit from referral to a dentist – preferably with a specialization in orofacial pain and dysfunction if available in the region. In addition to official TMD diagnoses, the results of this study also show a high prevalence of pain and non-painful symptoms during the clinical examination not resulting in an official diagnosis, particularly in the at-risk group. This could be an early sign of later TMD-pain complaints, [29] and further strengthens the suggestion to be alert to TMD-pain disorders in individuals at risk of RA. With these results, this study is the first to report on TMJ involvement in at-risk individuals.

The ERA group did not differ from the control group in prevalence of TMD pain. However, within the ERA group, seronegative patients more often had a TMD-pain diagnosis than seropositive patients. Seronegative patients were also more often categorized as being in remission based on the DAS28 score, possibly due to the longer time between RA diagnosis and participation in the study and thus better results of the pharmacological treatment for RA. Furthermore, both the DAS28 score and RAPID-3 score were not related to the prevalence of TMD pain. In only a few patients TMJ pain had subsided after treatment for RA. These results suggest that TMD pain occurs regardless of the general disease status, and could be associated with seronegative RA.

On the other hand, some cases indicate that the start of pharmacological treatment for RA could have lowered the prevalence of TMD in the ERA group; two patients reported familiar pain during the clinical examination and on the questionnaire, but not during the past 30 days, and three patients specifically mentioned experiencing less pain or even being pain free since the start of pharmacological treatment for RA. This is in accordance with the findings of Chin Jen Sem et al., [8] where prevalence of pain on palpation of the TMJ in a group of ERA patients decreased after the start of systemic RA treatment. Because four out of these five patients were seropositive ERA patients, the mechanism of TMJ involvement and thus the effect of systemic treatment on the TMJ might be seropositivity dependent. This corresponds to the findings of Alstergren et al., where TMJ pain on mandibular movement was mainly correlated to systemic factors in seropositive patients, but to local factors in seronegative patients. [30] Furthermore, seropositive patients had higher systemic inflammatory activity, but lower TMJ movement pain intensity, [30] which also corresponds to our findings.

In our study, mandibular movement capacity was not related to TMD pain, nor limited in ERA patients and at-risk individuals. In general, maximum mouth opening capacity can be limited in patients with TMD and is therefore often an outcome measure when evaluating TMD-treatment efficacy, [31] but our results do not confirm a relation between TMD pain and limited mouth opening for the present study population. For ERA patients, our results do correspond to an earlier study by Kroese et al., [32] where patients with ERA and established RA were compared: while TMJ pain was already present in ERA, reduced mouth opening capacity was found to be related to established RA.

We noted that for the vast majority of participants who reported possible sleep bruxism, also a probable bruxism was determined based on clinical findings. The prevalence of probable sleep bruxism did not differ between the three groups. However, probable sleep bruxism was found more often in participants with a TMD-pain diagnosis compared to participants without a TMD-pain diagnosis. These results suggest an association between sleep bruxism and TMD pain in the present study population. This corresponds to literature on other populations. [13,33,34]

Implications for practice

Based on our results, it is recommended to watch out for the possible presence of TMD pain in individuals at risk of developing RA and in patients with seronegative ERA. In the ERA group, TMD pain occurred regardless of general disease status. The few individuals that did seem to benefit from general RA treatment on TMD pain, reported that they experienced less pain or were pain free since the start of the pharmacological treatment. This suggests that an effect on TMD pain can be expected early, and thus screening for a possible TMD that needs additional treatment should start soon after the start of the pharmacological treatment.

In our study, the positive predictive value of the DC/TMD questionnaire was 68% for TMD-pain diagnoses. Only four questions need to be answered in order to screen for possible TMD pain, of which three do not have to be answered if the answer to the first question is negative. Therefore, the section about pain of the questionnaire [22] could be a quick, simple, and valid screening tool for rheumatologists or other health professionals in order to identify ERA patients or at-risk individuals that could benefit from further TMD examination and management.

Strengths and limitations

Strengths of this study include the extensive and standardized TMD examination according to the DC/TMD, the evaluation of bruxism as a possible associated factor, and the matching of the control group to the other two groups. However, there are also certain limitations to this study. Within the ERA group, some analyses were performed to compare seropositive and seronegative patients, and to compare patients with a DAS28 score higher or lower than 2.6. Although the results indicate some interesting differences, dividing the ERA group in subgroups consequently means lowering the number of subjects per group, which limits the power of the analyses and generalizability of the results. These results should therefore be interpreted with caution, and research on a larger group of ERA patients would be necessary to confirm the present findings.

Possible related factors to a TMD(-pain) diagnosis were tested for the total study population, in order to have total numbers that are substantial enough for statistical analysis. These analyses therefore do not provide information on the individual groups. For the same reason, results for myalgia and arthralgia were combined as TMD-pain disorders. Consequently, the results do not provide specific information on the nature of the TMD pain. However, independently of its nature, TMD pain is a relevant clinical outcome because it can negatively influence the oral health-related quality of life, most often on the subdomains of psychological discomfort and disability, and causing functional limitation. [35,36]

In this study, probable bruxism was diagnosed based on self-report and several clinical factors. This is a limitation of the study, since definite bruxism diagnoses were not established. Furthermore, the number of participants reporting awake bruxism was too small for statistical analyses. However, the diagnostic grading system for bruxism requires polysomnographic recording or electromyographic recording for a definite diagnosis of sleep or awake bruxism, respectively, [11,12] which are labour-intensive and costly procedures that are therefore only seldom applied in larger clinical studies. Furthermore, there is still much debate about the ideal way to assess sleep and awake bruxism, as acknowledged by the authors of the diagnostic grading system. [12] For future research including the assessment of awake bruxism, the use of Ecological Momentary Assessment [37,38] is recommended.

Conclusion

The results of this study provide a valuable insight into the prevalence and clinical characterization of TMJ involvement in patients with ERA and at-risk individuals. Based on these results, it is recommended to be alert to TMD-pain disorders in individuals at risk of developing RA and in patients with seronegative ERA. The DC/TMD symptom questionnaire is suggested as a useful tool for rheumatologists or other health professionals to screen for possible TMD pain in these groups. Individuals who might benefit from further TMD examination and management can then be identified and referred to a suitable dental healthcare provider, preferably with a specialization in orofacial pain and dysfunction. Sleep bruxism might be an important factor in the development of TMD pain in the study population.

References

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376:1094-1108.
2. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nat Rev Immunol. 2017;17:60-75.
3. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-1588.
4. Netherlands Institute for Health Services Research. Tabellenboek Reumatische aandoeningen in Nederland, 2016. Available from: https://www.nivel.nl/sites/ default/files/bestanden/Tabellenboek_Reumatische_aandoeningen_Nederland. pdf
5. Wolfe F, Katz RS, Michaud K. Jaw pain: its prevalence and meaning in patients with rheumatoid arthritis, osteoarthritis, and fibromyalgia. J Rheumatol. 2005;32:2421-2428.
6. Bono AE, Learreta JA, Rodriguez G, Marcos JC. Stomatognathic system involvement in rheumatoid arthritis patients. Cranio. 2014;32:31-37.
7. Lin Y-C, Hsu M-L, Yang J-S, Liang T-H, Chou S-L, Lin H-Y. Temporomandibular Joint Disorders in Patients with Rheumatoid Arthritis. Journal of the Chinese Medical Association. 2007;70:527-534.
8. Chin Jen Sem JP, van der Leeden M, Visscher CM, Britsemmer K, Turk SA, Dekker J, et al. Prevalence, Course, and Associated Factors of Pain in the Temporomandibular Joint in Early Rheumatoid Arthritis: Results of a Longitudinal Cohort Study. J Oral Facial Pain Headache. 2017;31:233-239.
9. van Boheemen L, van Schaardenburg D. Predicting Rheumatoid Arthritis in At-risk Individuals. Clin Ther. 2019;41:1286-1298.
10. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. J Oral Facial Pain Headache. 2014;28:6-27.
11. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, et al. Bruxism defined and graded: an international consensus. J Oral Rehabil. 2013;40:2-4.
12. Lobbezoo F, Ahlberg J, Raphael KG, Wetselaar P, Glaros AG, Kato T, et al. International consensus on the assessment of bruxism: Report of a work in progress. J Oral Rehabil. 2018;45:837-844.
13. Jimenez-Silva A, Pena-Duran C, Tobar-Reyes J, Frugone-Zambra R. Sleep and awake bruxism in adults and its relationship with temporomandibular disorders: A systematic review from 2003 to 2014. Acta Odontol Scand. 2017;75:36-58.
14. Kroese JM, Volgenant CMC, van Schaardenburg D, Loos BG, Crielaard W, Lobbezoo F. Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol. BDJ Open. 2020;6:7.
15. van Beers-Tas MH, Marotta A, Boers M, Maksymowych WP, van Schaardenburg D. A prospective cohort study of 14-3-3η in ACPA and/or RF-positive patients with arthralgia. Arthritis Research & Therapy. 2016;18:76.
16. Holla JFM, van Beers-Tas MH, van de Stadt LA, Landewe R, Twisk JWR, Dekker J, et al. Depressive mood and low social support are not associated with arthritis development in patients with seropositive arthralgia, although they predict increased musculoskeletal symptoms. RMD Open. 2018;4:e000653.
17. van Beers-Tas MH, Blanken AB, Nielen MMJ, Turkstra F, van der Laken CJ, Meursinge Reynders M, et al. The value of joint ultrasonography in predicting arthritis in seropositive patients with arthralgia: a prospective cohort study. Arthritis Res Ther. 2018;20:279.
18. van Riel PL, Renskers L. The Disease Activity Score (DAS) and the Disease Activity Score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol. 2016;34:S40-S44.
19. Fransen J, Creemers MC, Van Riel PL. Remission in rheumatoid arthritis: agreement of the disease activity score (DAS28) with the ARA preliminary remission criteria. Rheumatology (Oxford). 2004;43:1252-1255.
20. Pincus T, Bergman MJ, Yazici Y. RAPID3-an index of physical function, pain, and global status as “vital signs” to improve care for people with chronic rheumatic diseases. Bull NYU Hosp Jt Dis. 2009;67:211-225.
21. Ohrbach R (editor). Diagnostic Criteria for Temporomandibular Disorders: Assessment Instruments. Version 15 May 2016. Available from: www.rdc-tmdinternational.org.
22. Eke PI, Genco RJ. CDC Periodontal Disease Surveillance Project: background, objectives, and progress report. J Periodontol. 2007;78:1366-1371.
23. Ohrbach R, Gonzalez YM, List T, Michelotti A, Schiffman E. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD): Clinical Examination Protocol. Version 06 January 2014. Available from: www.rdc-tmdinternational.org.
24. Baad-Hansen L, Thymi M, Lobbezoo F, Svensson P. To what extent is bruxism associated with musculoskeletal signs and symptoms? A systematic review. J Oral Rehabil. 2019;46:845-861.
25. International network for orofacial pain and related disorders methodology (INfORM) DC-TMD examiner training. 2016. Available from: https://www.iadr.org/ INfORM/DC-TMD/Examiner-Training.
26. Wetselaar P, Lobbezoo F. The tooth wear evaluation system: a modular clinical guideline for the diagnosis and management planning of worn dentitions. J Oral Rehabil. 2016;43:69-80.
27. Nederlandse Vereniging voor Reumatologie. Richtlijn Medicamenteuze Behandeling van Reumatoïde artritis. 2020.
28. Poluha RL, Canales GT, Costa YM, Grossmann E, Bonjardim LR, Conti PCR. Temporomandibular joint disc displacement with reduction: a review of mechanisms and clinical presentation. J Appl Oral Sci. 2019;27:e20180433.
29. Ohrbach R, Bair E, Fillingim RB, Gonzalez Y, Gordon SM, Lim PF, et al. Clinical orofacial characteristics associated with risk of first-onset TMD: the OPPERA prospective cohort study. J Pain. 2013;14:T33-50.
30. Alstergren P, Fredriksson L, Kopp S. Temporomandibular joint pressure pain threshold is systemically modulated in rheumatoid arthritis. J Orofac Pain. 2008;22:231-238.
31. Calixtre LB, Moreira RF, Franchini GH, Alburquerque-Sendin F, Oliveira AB. Manual therapy for the management of pain and limited range of motion in subjects with signs and symptoms of temporomandibular disorder: a systematic review of

randomised controlled trials. J Oral Rehabil. 2015;42:847-861.
32. Kroese JM, Kopp S, Lobbezoo F, Alstergren P. TMJ Pain and Crepitus Occur Early Whereas Dysfunction Develops Over Time in Rheumatoid Arthritis. J Oral Facial Pain Headache. 2020;34:398-405.
33. Huhtela OS, Napankangas R, Joensuu T, Raustia A, Kunttu K, Sipila K. Self-Reported Bruxism and Symptoms of Temporomandibular Disorders in Finnish University Students. J Oral Facial Pain Headache. 2016;30:311-317.
34. Magalhaes BG, Freitas JLM, Barbosa A, Gueiros M, Gomes SGF, Rosenblatt A, et al. Temporomandibular disorder: otologic implications and its relationship to sleep bruxism. Braz J Otorhinolaryngol. 2018;84:614-619.
35. Dahlström L, Carlsson GE. Temporomandibular disorders and oral health-related quality of life. A systematic review. Acta Odontol Scand. 2010;68:80-85.
36. Papagianni CE, van der Meulen MJ, Naeije M, Lobbezoo F. Oral health-related quality of life in patients with tooth wear. J Oral Rehabil. 2013;40:185-190.
37. Osiewicz MA, Lobbezoo F, Bracci A, Ahlberg J, Pytko-Polonczyk J, Manfredini D. Ecological Momentary Assessment and Intervention Principles for the Study of Awake Bruxism Behaviors, Part 2: Development of a Smartphone Application for a Multicenter Investigation and Chronological Translation for the Polish Version. Front Neurol. 2019;10:170.
38. Zani A, Lobbezoo F, Bracci A, Ahlberg J, Manfredini D. Ecological Momentary Assessment and Intervention Principles for the Study of Awake Bruxism Behaviors, Part 1: General Principles and Preliminary Data on Healthy Young Italian Adults. Front Neurol. 2019;10:169.

Temporomandibular joint disorders: consequences

TMJ pain and crepitus occur early whereas dysfunction develops over time in rheumatoid arthritis

Johanna M. Kroese, Sigvard Kopp, Frank Lobbezoo, Per Alstergren

Published in: Journal of Oral & Facial Pain and Headache. 2020;34(4):398-405.
PMID: 33290445.

Chapter 04

ABSTRACT

Aims: To investigate inflammatory mediator levels in temporomandibular joint (TMJ) synovial fluid (SF), blood and clinical TMJ symptoms in relation to general and TMJ symptom duration in patients with rheumatoid arthritis (RA).

Methods: Examination of 80 TMJs (68 patients; median age 55 years; 85% females) included TMJ pain on rest, maximum mouth opening and palpation, jaw movement capacity, number of painful movements, crepitus, and degree of anterior open bite. Levels of TNF, TNFsRII, IL-1β, IL-1ra, IL-1sRII, and 5-HT in TMJ SF and blood, systemic disease activity, and duration of general and TMJ symptoms were assessed. General symptom duration ≤2 years was considered early RA.

Results: TMJ symptoms predominantly developed within five years following general symptom onset. Logistic regression analysis showed that number of involved joints, general pain, maximum mouth opening, anterior open bite, and TNF plasma levels combined explained 46% of the distinction between early and established RA. Furthermore, TMJ pain on rest and maximum mouth opening, contralateral laterotrusion, painful movements, crepitus, and SF TNF levels combined explained 35%. In these analyses, higher general pain and maximum mouth opening, TMJ pain on maximum mouth opening, and crepitus were associated with early RA.

Conclusion: This study indicates that TMJ pain and crepitus in RA usually occur within two years following general symptom onset. Pain-related dysfunction and structural changes develop with time. TNF in plasma and TMJ SF are associated with this development. This makes early (clinical) recognition of pain and inflammation important, enabling early treatment to minimize later irreversible damage.

Introduction

Synovial inflammation is one of the key characteristics of rheumatoid arthritis (RA). Many of the changes that occur in the inflamed synovium can be observed in the synovial fluid (SF). Predominantly, SF contains cytokines that play important roles in the inflammatory response. [1] Pro-inflammatory cytokines have even been found present prior to clinical symptom onset. [2] Also, the levels of several cytokines in early inflammatory arthritis seem to be higher than in patients with established RA. [3]

RA symptoms usually debut bilaterally in small peripheral joints, [4] but may involve other joints as well, including the temporomandibular joint (TMJ). [5] TMJ pain as well as TMJ cartilage and bone tissue destruction have been strongly associated with increased SF levels of the cytokines tumor necrosis factor (TNF) and interleukin-1β (IL-1β). [6,7]

Because of the advantages of early diagnosis and treatment on disease outcomes, identifying patients with early RA is of great importance. [8] Insight into a possible difference between patients with early and established RA regarding inflammatory mediators in TMJ SF and clinical TMJ symptoms could be crucial in order to develop better diagnostic procedures and management of TMJ involvement in RA. Rheumatologists and dentists share the possibility to diagnose and manage RA cases with TMJ involvement at an early stage to improve prognosis.

Therefore, the aim of the current study was to investigate inflammatory mediator levels in TMJ SF, blood and clinical TMJ symptoms in relation to duration of local and general symptoms in patients with RA.

Materials and Methods

Patients

A total of 68 patients, 58 women and 10 men, with RA according to the 1987 classification criteria of the American College of Rheumatology, were included in this study. Fifty-one patients (75%) were positive for the rheumatoid factor (RF). The patients were referred to the specialist clinic for Orofacial Pain and Jaw Function, mainly because of TMJ pain, by rheumatologists in the Stockholm area, Sweden. The patients were included and examined between 1993 and 2007. Systemic pharmacological treatment of the general disease was provided by the referring rheumatologists. For a subset of 15 patients, specific data on pharmacological treatment were available: nine patients received a Disease

Modifying Anti-Rheumatic Drug (DMARD) – the majority being Methotrexate – of which four in combination with biological anti-TNF treatment, two patients received anti-TNF treatment only, and four patients only received an NSAID. For the other 53 patients, no specific data is available. However, when taking into account the period of inclusion for this study, all participants were presumably treated by either an NSAID, DMARD, anti-TNF, or a combination. [9] Table 1 describes the included patient sample.

This project was approved by the regional ethical committee at Karolinska Institutet, Stockholm, Sweden (176/91; 310/97; 142/02; 03-2004) according to the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Table 1. Demographic data for 68 patients with Rheumatoid Arthritis

		Percentile		
	Median	**25th**	**75th**	**n**
Age (y)	55	42	64	68
Gender (M/F)				10/58
Duration of general joint symptoms (y)	7	3	21	68
Duration of local TMJ symptoms (y)	3	1	10	60
Time between onset of general and TMJ symptoms (y)	4	0	13	60
RF positivity, n (%)				51 (75)
Erythrocyte sedimentation rate	28	16	37	67
C-reactive protein	11	0	21	67
Thrombocyte particle count	305	280	374	54

N = number of observations, TMJ = temporomandibular joint, RF = rheumatoid factor

Assessment of subjective symptoms and clinical signs

To assess aspects of the degree of general disease, patients were asked about musculoskeletal complaints in nine joint regions besides the TMJ (neck, shoulders, elbows, hands, upper back, lower back, hips, knees, and feet), resulting in a score from 0 to 9. Pain intensity of general symptoms was assessed using either a visual analogue scale (VAS) or a numerical rating scale (NRS) with the end points “no pain” (score 0) and “worst pain ever experienced” (score 10). Minimal influence on the results is expected by the mix of visual and numerical scales due to the high correspondence between the two types of scales. [10]

Local TMJ pain intensity at rest was assessed using either the VAS or NRS. Tenderness on digital palpation reported by the patient or a palpebral reflex, was recorded as present or absent for the lateral aspect of the TMJ using 20 N pressure.

Maximum voluntary mouth opening was measured in millimeters between the right central incisors, and pain intensity on mouth opening was recorded on either the VAS or NRS. Number of painful mandibular movements (maximum voluntary mouth opening, protrusion, ipsilateral and contralateral laterotrusion; maximum score = 4) was counted for each TMJ.

Pressure pain threshold (PPT) was measured by linearly increasing pressure of approximately 50kPa/s to the lateral aspect of the TMJ, with an algometer with a 1cm² rubber tip. The PPT was defined as the minimum pressure needed to evoke a painful sensation recognizable by the patient.

Crepitus was recorded as present if crepitus was palpable or audible in at least one of three maximum mouth opening movements.

The degree of anterior open bite (AOB) was used as a clinical marker of the degree of cartilage and bone destruction in the TMJ and it was assessed by recording of the occlusal contacts on each side upon hard biting on occlusal foil in intercuspid position (2 x 8 µm, Occlusions-Prüf-Folie, GHM Hanel Medizinal, Nürtingen, Germany). The following scores were used in the assessment of AOB on each side: 0 = occlusal contacts including the canine, 1 = no contacts anterior to the first premolar, 2 = no contacts anterior to the second premolar, 3 = no contacts anterior to the first molar, 4 = no contacts anterior to the second molar and 5 = no occlusal contact. The sum of the scores on the right and left side was used in the analysis as an estimation of the degree of AOB. None of the patients in our study was edentulous and the score thus ranged from 0 to 9. Score 9 (4+5) means that only one contact between two opposing teeth exists on one side.

All clinical examinations were performed by two experienced examiners (PA, SK), and the two examiners were calibrated regularly throughout the years in order to prevent drift. The calibration was both theoretical and clinical.

Temporomandibular joint synovial fluid sampling

All synovial fluid samples were obtained by the same two experienced and specially trained operators (PA, SK) according to Alstergren *et al*. as follows. [11-13] Anesthesia of the TMJ was achieved by blocking of the auriculotemporal nerve with 2.0 mL Xylocain® (lidocaine 2%, Astra, Södertälje, Sweden). The TMJ was punctured with a standard disposable needle (diameter = 0.6 mm) inserted into the posterior part of the upper joint compartment. TMJ synovial fluid samples were obtained by washing the joint cavity with saline, using a push-and-pull technique performed with two syringes, one used for the washing solution to be injected and the other for aspiration. The syringes were connected to

the arthrocentesis needle by a three-way stopcock. The injected washing solution consisted of 78% saline (NaCl 9 mg/mL, Kabi Pharmacia, Uppsala, Sweden) and 22% Behepan® (hydroxocobalamin, 1 mg/mL, Kabi Pharmacia, Uppsala, Sweden). It was injected slowly into the joint cavity in 1 mL portions and aspirated after approximately 20 s. This procedure was repeated three times, and the total washing solution volume used was 4 mL. The Behepan® was included in order to measure the amount of synovial fluid in the aspirate. The absorbance of the aspirate and a sample of unused washing solution were compared in a spectrophotometer (Shimadzo UV-160A, Shimadzo Corp., Tokyo, Japan) at 350 nm with a capillary tube system consisting of a capillary tube of quartz (3 µL/sample) and a capillary tube holder (Shimadzo Corp., Tokyo, Japan). The detection limit regarding dilution of the washing solution by synovial fluid in the aspirate by this method is 0.9 %.
The true synovial fluid concentrations were then calculated using the formula:

$$C_{SF} = \frac{C_{Asp}}{\left(1 - \frac{Abs_{Asp}}{Abs_{wash}}\right)}$$

where C_{SF} = synovial fluid concentration, C_{Asp} = aspirate concentration, Abs_{Asp} = aspirate absorbance, and Abs_{Wash} = washing solution absorbance.

During and after the arthrocentesis, blood contamination of the aspirate was estimated visually according to the following scale: no visible blood contamination, hardly visible blood contamination, clearly visible blood contamination, and blood-like appearance of the aspirate. After aspiration, the weight of the sample was immediately measured by a balance (JW-120, Adam Equipment Co, Milton Keynes, UK) and the sample was then centrifuged (1500 g for 10 min in 4°C). Hemolysis was then recorded as absent or present. Twelve µL of the supernatant, i.e., 4 capillary tubes, was used for absorbance measurement. The aspirate and washing solution absorbances were compared and a dilution factor ($Absorbance_{Aspirate}$ / $Absorbance_{Washing\ solution}$) was calculated for each sample. The same procedures, apparatus and protocols were used for analysis of absorbance as well as for the sample handling throughout the study period.

Sample quality criteria
In order to make sure only high-quality samples were used in the statistical analysis, included samples had to meet the sample quality criteria according to Alstergren et al., [13] which exclude samples with hemolysis, clearly visible blood contamination, aspirate weight <0.5 g, and a dilution factor of >0.98.

Blood sampling
Venous blood was collected and used for determination of rheumatoid factor level, erythrocyte sedimentation rate, serum level of C-reactive protein, as well as plasma or serum levels of inflammatory mediators. Rheumatoid factor titers below 15 IE/mL and C-reactive protein levels below 10 mg/L were considered as zero values according to the standard procedures of the accredited laboratory at the Department of Clinical Chemistry at Karolinska University Hospital, Huddinge, Sweden.

Analysis of mediators
The TMJ synovial fluid and blood plasma concentrations of TNF, TNF soluble receptor II (TNFsRII), IL-1β, IL-1 receptor antagonist (IL-1ra), IL-1 soluble receptor II (IL-1sRII), and serotonin (5-HT) were determined using commercially available enzyme-linked immunoassays, in which highly specific antibodies were used to detect the mediators (TNF, TNFsRII, IL-1β, IL-1ra, IL-1sRII, R&D Systems, Minneapolis, MN USA; Serotonin: Serotonin EIA-kit, Immunotech A Coulter Company, Marseille, France). The serotonin assay for SF concentrations was modified to be applicable at concentrations between 1.6 and 5000 nmol/L. To compensate for hydroxocobalamin interaction with the assay, the synovial fluid aspirates were read against a standard curve with hydroxocobalamin included [11,13]. The small hydroxocobalamin interaction was completely compensated for by this procedure.

Although data was collected over a period of several years, possible drift in performance is expected to be of minor and insignificant extent since the commercially available assays are quality controlled by the manufacturer in order to ensure consistent performance. We used the same assay for each mediator during the study period.

Temporomandibular joint arthritis
Probable and definite TMJ arthritis were defined according to Alstergren et al. [14] In the present study, TMJ arthritis was considered present if the joint fulfilled at least the criteria for probable arthritis, being a combination of pain on maximum mouth opening and contralateral laterotrusion of less than 8 mm. These simple measures are not trend-dependent or influenced by new insights or techniques since exactly the same clinical procedures were used, and therefore the criteria, although published in 2018, are applicable to the data currently described as well.

Statistics

Non-parametric statistics were used throughout the study, because the majority of variables was either not normally distributed or not measured on an interval scale. For descriptive statistics, median values and 25th/75th percentiles are presented.

For joint-related variables where there were bilateral data, the joints were considered as two separate statistical units. This enabled statistical analysis that tested the combination of local SF levels of inflammatory mediators and local TMJ symptoms from the same joint in relation to general and TMJ symptom duration.

To calculate the significance of correlations, the duration of general and local TMJ symptoms in years was used as a scale variable, and variables were tested with the Spearman's ranked correlation test.

For logistic regression analysis based on general symptom duration, patients were grouped in either early RA, defined as general symptom duration of two years or less, or established RA. This dichotomized variable was then used as dependent variable in the statistical model. Initially, all patient- or joint-related variables from the clinical, blood and SF examinations were entered into the logistic regression as independent variables. A stepwise backwards procedure was applied, where the independent variable with the least predictive value was eliminated from the model until the remaining model reached significance. For logistic regression analysis on local TMJ symptom duration, the same cut-off of two years was used to distinguish between early and persisting TMJ symptoms. This dichotomized variable was then used as dependent variable in the statistical model. For the independent variables the same stepwise backwards procedure was applied.

A probability level of $P<0.05$ was considered as significant.

Results

Clinical examination including laboratory variables

Table 2 shows the investigated clinical, synovial fluid, and blood variables. Synovial fluid samples were collected from 80 joints. Twelve patients had bilateral samples. Detectable levels of TNF were found in 14 out of 58 (24%) samples and detectable levels of IL-1β were found in 12 out of 75 (16%) samples. Fifty-four joints were diagnosed with either probable or definite arthritis.

Symptom duration

Figure 1 shows the distribution of differences in years of onset of general and TMJ symptoms. The majority of patients developed TMJ symptoms within five years or even prior to the onset of general symptoms (Fig. 1).

There was a positive correlation between the duration of general symptoms and duration of TMJ symptoms (r_s = .638, n = 60, p < .001; Fig. 2), where duration of TMJ symptoms was consistently shorter.

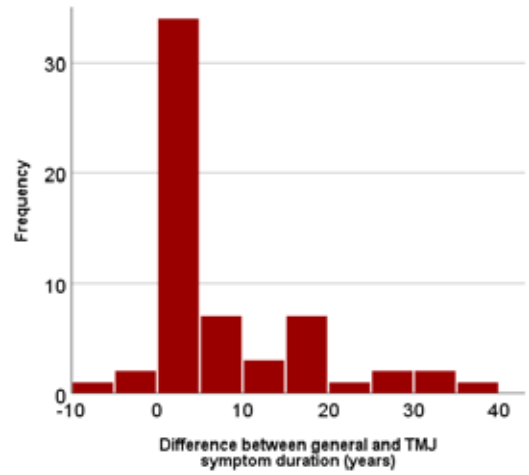


Figure 1. Histogram showing the distribution of difference in duration of general and temporomandibular joint (TMJ) symptoms in 60 patients with rheumatoid arthritis.

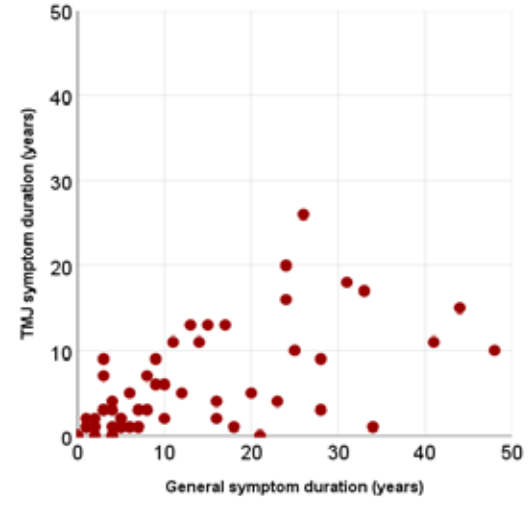


Figure 2. Scatterplot showing the relation between duration of general symptoms and duration of temporomandibular joint (TMJ) symptoms in 60 patients with rheumatoid arthritis (rs = .638, n = 60, p < .001).

Table 2. Clinical and blood variables in 80 TMJ's of 68 patients with Rheumatoid Arthritis.

	Median	Percentile 25th	Percentile 75th	n
Variables on individual level				
General disease activity				
Number of painful joint regions	6	4	7	38
Pain intensity	4	2	6	49
Plasma IL-1β	0	0	2925	62
Plasma TNF	13	9	28	52
TMJ related findings				
Maximum mouth opening, mm.	41	35	46	68
Degree of anterior open bite	0	0	2	66
Variables on joint level				
TMJ pain intensity at rest	2	0	5	62
TMJ pain intensity upon mouth opening	0	0	4	61
Number of painful movements	1	0	3	60
Pain to palpation, n (%)				17 (21)
Pressure pain threshold				
Glabella	274	177	379	52
TMJ	145	99	298	77
Laterotrusion to the contralateral side	9	7	11	79
Crepitus, n (%)				26 (33)
Inflammatory mediators in SF				
TNF	0	0	5	58
IL-1β	0	0	0	75
IL-1sRII	1372	628	3790	29
IL-1ra	743	280	2003	28
5-HT	31	0	450	32
Joints with arthritis, n (%)				54 (67)

N = number of observations, TMJ = temporomandibular joint, IL-1β = interleukin-1β, TNF = tumor necrosis factor, SF = synovial fluid, IL-1sRII = interleukin-1 soluble receptor II, IL-1ra = interleukin-1 receptor antagonist, 5-HT = serotonin

Relation between duration of symptoms versus clinical, blood, and temporomandibular joint synovial fluid variables

Table 3 shows the significant correlations between duration of general and TMJ symptoms and the investigated variables.

When comparing patients with early RA to patients with established RA, the combination of number of involved joints, general pain intensity, maximum voluntary mouth opening, degree of anterior open bite, and plasma levels of TNF explained 46% of the distinction between the groups (p = .012). Higher general pain intensity and greater maximum mouth opening capacity were associated with early RA, whereas higher number of involved joints, higher degree of anterior open bite, and higher plasma levels of TNF were associated with established RA. Furthermore, the combination of current TMJ pain intensity at rest, pain intensity on maximum voluntary mouth opening, laterotrusion to the contralateral side, number of painful movements, crepitus, and SF levels of TNF explained 35% of the difference between early RA and established RA (p = .019). Here, a higher TMJ pain intensity on maximum mouth opening and crepitus were associated with early RA.

Table 3. Relation between duration of general and TMJ symptoms and clinical, blood and synovial fluid variables in patients with Rheumatoid Arthritis.

	rs	n	*P*
Duration of general symptoms			
Plasma IL-1sRII	0.50	21	0.022
Number of involved joints	0.46	38	0.040
Mouth opening capacity	-0.27	68	0.024
TMJ pain intensity at rest	0.26	62	0.041
Synovial fluid 5-HT	0.36	32	0.041
Duration of local TMJ symptoms			
Plasma IL-1ra	-0.55	24	0.006
Number of involved joints	0.38	32	0.030
Anterior open bite	0.29	58	0.025

TMJ = temporomandibular joint, rs = Spearman rank-order coefficient, n = number of observations, P = probability, IL-1sRII = interleukin-1 soluble receptor II, 5-HT = serotonin, IL-1ra = interleukin-1 receptor antagonist

Besides comparing patients based on general symptom duration, a similar analysis was performed to compare patients with short and long duration of TMJ symptoms. In this analysis, the combination of TMJ pain intensity on maximum mouth opening, number of painful jaw movements, and SF levels of TNF explained 15% of the distinction between the groups (p = .041). Higher TMJ pain intensity on maximum mouth opening was associated with short duration of TMJ symptoms, whereas higher number of painful jaw movements and higher SF levels of TNF were associated with persisting TMJ symptoms.

Discussion

This study indicates that in RA patients with TMJ symptoms, the onset of these symptoms predominantly occurs in the first five years following general symptom onset. Early RA seems to be associated with high general and TMJ pain intensity and early TMJ cartilage degradation. On the other hand, established RA seems to be associated with high general and TMJ disease activity, TMJ cartilage and bone tissue destruction, and high systemic and local TNF levels, and at the same time reduced TMJ function. In addition, short duration of TMJ symptoms seems to be associated with TMJ pain but at the same time with low TMJ disease activity and low local TNF levels.

In our study, early general RA symptoms were associated with high general pain intensity, TMJ pain intensity on maximum mouth opening, TMJ crepitus, and greater maximum mouth opening capacity, but also with low number of involved joints, low degree of anterior open bite, and low plasma levels of TNF. Both general and TMJ pain intensity thus seem to be higher in early RA, which is consistent with the higher systemic inflammatory activity usually found in early RA. [2] Also, early RA was found to be associated with low number of involved joints. This is likely because involvement of joints increases with disease duration and is preceded by increased systemic inflammatory activity. [15] High TMJ pain intensity but also low number of painful jaw movements and greater maximum mouth opening capacity were associated with early RA. TMJ pain on mouth opening may therefore be regarded as an early sign of TMJ involvement in RA, but does not seem to occur in conjunction with the lower maximum mouth opening capacity in established RA.

TMJ crepitus was associated with early RA, suggesting that TMJ cartilage and perhaps also bone tissue destruction occurs early and in parallel with TMJ pain. This is in accordance with the findings of Hajati et al., where radiographic signs of bone tissue resorption of the TMJ were found in the majority of an early RA cohort [16] and presence of crepitus could predict TMJ bone tissue resorption in early RA. [17] Furthermore, both TMJ pain on maximum mouth opening and crepitus have been found to be associated with TMJ arthritis, [14] which may explain this finding. TNF in plasma and TMJ SF have previously been associated with TMJ pain and tissue destruction, [18,19] but in our present study both plasma and TMJ SF were found to be low in early RA. This was interesting but somewhat surprising since TNF is known to be involved also in early RA, not the least by the potent treatment effects of anti-TNF therapy also in early RA. [2,20] Certainly, the immune system reactions involved in RA are highly complex and involve many other inflammatory mediators, but TNF is an important aspect of joint inflammation. Patients with early inflammatory arthritis, who subsequently developed RA, have been found to have a distinct but transient SF cytokine profile, [3] indicating a change in cytokine profile with the duration of the disease. In established RA, TNF has been associated with TMJ cartilage and bone tissue destruction, as assessed by computerized tomography as well as the potential clinical consequence ‘anterior open bite’. [7] Indeed, anterior open bite was associated with established RA in the present study as well.

Degree of anterior open bite was used as a clinical sign of TMJ tissue destruction, which may be a severe clinical problem for the patient as well as the dentist. The degree of anterior open bite was higher in established RA, supporting a progressive disease course. Our findings of both early tissue damage and a progressive disease course are supported by the findings of Uchiyama et al., where a prevalence of 69% was found for bone changes as assessed by magnetic resonance imaging in RA patients with a disease duration of <5 years, and the increasing number of bony changes was significantly correlated with the duration of the general disease. [21] The progressive disease course, together with the higher TMJ pain intensity and presence of crepitus in early RA, further strengthens the obvious need for early detection of TMJ involvement in RA. Furthermore and from a functional point-of-view, the degree of anterior open bite and the lower maximum mouth opening capacity found in established RA suggest that early recognition and treatment of TMJ inflammation is important.

In our study, TMJ symptoms occurred close to the onset of general symptoms for most patients, which has been shown before. [22] In our study, for three patients (4%), the TMJ symptom onset was before the onset of general symptoms. The duration of general symptoms was strongly associated with duration of TMJ symptoms, which further corroborates that TMJ involvement occurs early in the general disease course. The strong relation between general and TMJ duration of symptoms also explains the similar findings in the logistic regression analysis in both early general and early TMJ symptoms cases.

The limitations of this study that may influence the generalizability and conclusions of the results, further discussed below, include limitations in how well the patients could recall the onset of general and TMJ symptoms, the fact that the patients were referred to the clinic by rheumatologists because of TMJ symptoms, and the seropositivity was determined only by presence of RF. In addition, a limited number of samples had detectable SF concentrations or sufficient sample volume to analyze all inflammatory mediators of interest, bilateral data was available in a number of, but not all, cases, and the medication profile does not fully correspond to current guidelines.

The patients were asked to recall their debut year of general and TMJ symptoms. For some patients, particularly patients with long duration of symptoms, recollection of debut year may be difficult. However, we consider the data for patients with shorter duration of symptoms to be sufficiently accurate. Limited influence is therefore expected when using a cut-off point of two years when allocating patients to groups of early and longer symptom duration.

The patients were referred to the specialist clinic by rheumatologists because of TMJ complaints. The study population is thus not fully representative of the entire RA population. However, the aim of this study was not to report on prevalence or incidence of TMJ involvement in RA in the population. The paper does, on the other hand, give an insight in the characteristics of TMJ symptoms in relation to symptom duration.

The patients were allocated to groups based on duration of symptoms rather than duration of RA diagnosis. Actually, the duration of symptoms was of primary interest in our study, since it adds to our understanding of when in the disease course the TMJ symptoms debut. Furthermore, our study focusses on early RA including the period preceding RA diagnosis, just as can be found in many recent publications. [23]

Seropositivity was determined solely by presence or absence of RF. The current determination of seropositivity includes assessment of anti-citrullinated protein antibody (ACPA) levels. At the time when the data for this study was collected, ACPA assays were not available. However, the proportions of RF seropositivity and seronegativity in the study sample (75% seropositivity) very well resemble the general RA population, where 50-80% were reported to be positive for RF, ACPA, or both – with most ACTA-positive patients also being positive for RF. [24]

There were limited number of samples with detectable SF concentrations of TNF and IL-1β and the majority of samples had insufficient sample volume to analyze all inflammatory mediators of interest, which limits our analysis based on SF content. However, all included samples fulfilled the sample quality criteria, ensuring high quality SF data that we consider of great importance.

We included data from both joints for some, but not all, individuals, which makes the analysis of data a statistical challenge. When comparing joint-related variables, we used each joint as one statistical unit. Otherwise, we used the individual as a unit. The alternative would be to use only one joint for each individual, but this would mean losing valuable data and we believe that there is no justifiable way to choose which joint to use.

For most patients, no specific data on the pharmacological treatment were available, which is a limitation of the study to some degree. It is therefore not feasible to analyze a possible confounding effect of the RA medication on the investigated variables. Furthermore, the medication profiles of the patients do not fully correspond to current guidelines, because at the time of data collection the systemic pharmacological treatment did not yet include biologics. Developments in treatment strategies over the last decades have substantially changed the course of RA, nowadays frequently resulting in remission of the disease. [25,26] The possible effects of this improved systemic RA treatment on the TMJ would be an interesting topic for research. However, the aim of this study was to specifically investigate patients that have TMJ symptoms, despite systemic treatment or not, rather than investigating prevalence of TMJ symptoms in the total RA population or effects of systemic treatment on the TMJ. Although information on systemic treatment could complement analysis, we therefore argue that the lack of it does not devaluate the results that concern our aim substantially. Furthermore, recent studies still report a high prevalence of TMJ involvement in RA. [27,28] This suggests that, despite the greatly improved treatment modalities, the TMJ remains a joint that is important to monitor.

Conclusion

This study indicates that TMJ pain and crepitus in RA usually occur early in the disease, i.e., within two years following general symptom onset. Pain-related dysfunction worsens and structural changes develop with time, and presence of TNF in plasma and TMJ SF is associated with this development. This presumably makes early (clinical) recognition of pain and inflammation important, enabling early treatment to minimize later irreversible damage.

Acknowledgements

The authors wish to thank the laboratory technicians Karin Trollsås, Agneta Gustafsson, and Kari Eriksson for their tremendous work.

References

1. Veale DJ, Orr C, Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. Semin Immunopathol. 2017;39:343-354.
2. Ridgley LA, Anderson AE, Pratt AG. What are the dominant cytokines in early rheumatoid arthritis? Curr Opin Rheumatol. 2018;30:207-214.
3. Raza K, Falciani F, Curnow SJ, Ross EJ, Lee CY, Akbar AN, et al. Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin. Arthritis Res Ther. 2005;7:R784-795.
4. Kourilovitch M, Galarza-Maldonado C, Ortiz-Prado E. Diagnosis and classification of rheumatoid arthritis. J Autoimmun. 2014;48-49:26-30.
5. Savtekin G, Sehirli AO. Rheumatoid arthritis in temporo-mandibular joint: A review. Niger J Clin Pract. 2018;21:1243-1246.
6. Alstergren P, Ernberg M, Kvarnstrom M, Kopp S. Interleukin-1beta in synovial fluid from the arthritic temporomandibular joint and its relation to pain, mobility, and anterior open bite. J Oral Maxillofac Surg. 1998;56:1059-1065; discussion 1066.
7. Nordahl S, Alstergren P, Kopp S. Tumor necrosis factor-alpha in synovial fluid and plasma from patients with chronic connective tissue disease and its relation to temporomandibular joint pain. J Oral Maxillofac Surg. 2000;58:525-530.
8. Heidari B. Rheumatoid Arthritis: Early diagnosis and treatment outcomes. Caspian J Intern Med. 2011;2:161-170.
9. Upchurch KS, Kay J. Evolution of treatment for rheumatoid arthritis. Rheumatology (Oxford). 2012;51 Suppl 6:vi28-36.
10. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, et al. Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review. J Pain Symptom Manage. 2011;41:1073-1093.
11. Alstergren P, Appelgren A, Appelgren B, Kopp S, Lundeberg T, Theodorsson E. Determination of temporomandibular joint fluid concentrations using vitamin B12 as an internal standard. Eur J Oral Sci. 1995;103:214-218.
12. Alstergren P, Appelgren A, Appelgren B, Kopp S, Nordahl S, Theodorsson E. Measurement of joint aspirate dilution by a spectrophotometer capillary tube system. Scand J Clin Lab Invest. 1996;56:415-420.
13. Alstergren P, Kopp S, Theodorsson E. Synovial fluid sampling from the temporomandibular joint: sample quality criteria and levels of interleukin-1 beta and serotonin. Acta Odontol Scand. 1999;57:16-22.
14. Alstergren P, Pigg M, Kopp S. Clinical diagnosis of temporomandibular joint arthritis. J Oral Rehabil. 2018;45:269-281.
15. Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. Biomed Pharmacother. 2017;92:615-633.
16. Hajati AK, Alstergren P, Nasstrom K, Bratt J, Kopp S. Endogenous glutamate in association with inflammatory and hormonal factors modulates bone tissue resorption of the temporomandibular joint in patients with early rheumatoid arthritis. J Oral Maxillofac Surg. 2009;67:1895-1903.
17. Hajati AK, Nasstrom K, Alstergren P, Bratt J, Kopp S. Temporomandibular joint bone tissue resorption in patients with early rheumatoid arthritis can be predicted by joint crepitus and plasma glutamate level. Mediators Inflamm. 2010;2010:627803.
18. Alstergren P, Kopp S. Insufficient endogenous control of tumor necrosis factor-alpha contributes to temporomandibular joint pain and tissue destruction in rheumatoid arthritis. J Rheumatol. 2006;33:1734-1739.
19. Ahmed N, Petersson A, Catrina AI, Mustafa H, Alstergren P. Tumor necrosis factor mediates temporomandibular joint bone tissue resorption in rheumatoid arthritis. Acta Odontol Scand. 2015;73:232-240.
20. Emery P, Bingham CO, 3rd, Burmester GR, Bykerk VP, Furst DE, Mariette X, et al. Certolizumab pegol in combination with dose-optimised methotrexate in DMARD-naive patients with early, active rheumatoid arthritis with poor prognostic factors: 1-year results from C-EARLY, a randomised, double-blind, placebo-controlled phase III study. Ann Rheum Dis. 2017;76:96-104.
21. Uchiyama Y, Murakami S, Furukawa S. Temporomandibular joints in patients with rheumatoid arthritis using magnetic resonance imaging. Clin Rheumatol. 2013;32:1613-1618.
22. Tegelberg A, Kopp S. Subjective symptoms from the stomatognathic system in individuals with rheumatoid arthritis and osteoarthrosis. Swed Dent J. 1987;11:11-22.
23. Deane KD, Holers VM. The Natural History of Rheumatoid Arthritis. Clin Ther. 2019;41:1256-1269.
24. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376:1094-1108.
25. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet. 2007;370:1861-1874.
26. Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017;389:2338-2348.
27. Kurtoglu C, Kurkcu M, Sertdemir Y, Ozbek S, Gurbuz CC. Temporomandibular disorders in patients with rheumatoid arthritis: A clinical study. Niger J Clin Pract. 2016;19:715-720.
28. González-Chávez SA, Pacheco-Tena C, Campos Torres RM, Quiñonez-Flores CM, Reyes-Cordero G, Caraveo Frescas TJ. Temporomandibular and Odontological Abnormalities in Patients with Rheumatoid Arthritis. Reumatol Clin. 2020;16:262-271.

Temporomandibular joint disorders: treatment

Corticosteroid injections in the temporomandibular joint temporarily alleviate pain and improve function in rheumatoid arthritis

Johanna M. Kroese, Sigvard Kopp, Frank Lobbezoo, Per Alstergren

Published in: Clinical Rheumatology. 2021;40(12):4853-4860.
PMID: 34287722.

Chapter 05

ABSTRACT

Objectives: To evaluate the effect of corticosteroid injections in the painful temporomandibular joint (TMJ) of patients with rheumatoid arthritis (RA) in relation to systemic inflammatory activity.

Method: Examination of 35 patients (median age 54 years; 89% female) included maximum mouth opening capacity, degree of anterior open bite (AOB), TMJ-pain intensity at rest, and crepitus. Serum levels of rheumatoid factor (RF), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serotonin, and plasma levels of interleukine-1β (IL-1β) were determined. Out of the 70 examined joints, 53 joints received a corticosteroid (methylprednisolone) injection after the clinical examination at baseline (T0). The examination was repeated for all patients at T1 (median 3.1 weeks after T0), and for 21 patients at T2 (median 6.3 weeks after T1), of whom 20 patients received a second injection at T1.

Results: Maximum mouth opening capacity significantly increased, and TMJ-pain intensity significantly decreased between T0 and T1, but these improvements were no longer present at T2. No differences were found in AOB between the time points. Of the joints that received an injection at T0, 19 joints had pretreatment crepitus, which resolved in eight joints at T1. No correlations were found between the change in mouth opening capacity or TMJ pain intensity and ESR, CRP, serotonin, or IL-1β.

Conclusions: Methylprednisolone injections in the TMJ alleviate pain and improve mouth opening capacity for approximately three weeks, allowing patients to perform jaw exercises during this timeframe of temporary relief. It thus seems useful for the short-term management of TMJ involvement in RA.

Introduction

Rheumatoid arthritis (RA) is an auto-immune disease affecting the synovial joints. [1] RA symptoms usually start bilaterally in small peripheral joints, [2] but may involve other joints as well, including the temporomandibular joint (TMJ). [3] When the TMJ is involved, pain usually occurs within two years following general disease symptom onset, while pain-related dysfunction and structural changes develop with time. Early recognition and treatment are thus recommended to minimize irreversible damage. [4] TMJ pain can also negatively influence the oral health-related quality of life, [5] a subjective measure of disease burden, further supporting the need for treatment.

As recommended by the European League Against Rheumatism (EULAR), standard management of RA consists of systemic pharmacological treatment with disease-modifying antirheumatic drugs (DMARDs). [6] Occasionally, corticosteroid injections are used to alleviate pain and reduce swelling in joints that do not, or insufficiently respond to systemic treatment or experience a local flare-up. The most commonly injected joint is the knee, usually resulting in pain relief for approximately eight weeks, but other joints can be injected as well. [7]

In people with arthrogenous TMJ pain, corticosteroid injections were shown to be an effective method for pain reduction. [8] In patients with RA, a positive effect on function and subjective complaints was also found. [9] The clinical outcome of corticosteroid injections, in patients with several rheumatic diseases combined, was related to pretreatment synovial fluid concentrations of tumor necrosis factor-alpha [10] and serotonin, [11] as well as pretreatment systemic concentrations of serotonin. [11] However, specific data on patients with RA is limited.

Therefore, the aim of the current study was to evaluate the clinical effect of corticosteroid injections in the painful TMJ of patients with RA, which were performed as a part of routine care, in relation to systemic inflammatory activity. We hypothesize improvement of pain and, consequently, improvement of function after treatment with corticosteroid injections. Further, we hypothesize that lower pretreatment systemic inflammatory activity, as assessed by CRP and ESR, and lower pretreatment levels of serotonin and Interleukin-1β result in better treatment effects on TMJ pain and function.

Materials and Methods

Patients

A total of 35 patients, 31 woman and 4 men, with RA according to the 1987 classification criteria of the American College of Rheumatology, [12] were included in this study. The patients were referred to the specialist clinic for Orofacial Pain and Jaw Function (Karolinska Institutet, Institution of Odontology, Department of Clinical Oral Physiology, Huddinge, Sweden) by rheumatologists in the Stockholm area, Sweden. The patients were included and examined between 1990 and 2006. Systemic pharmacological treatment of the general disease was provided by the referring rheumatologists; no specific data is available. The period of data collection was mostly before the introduction of biologics, which means that the medication profiles do not fully correspond to current guidelines. However, the aim of this study was to measure the effect of local treatment with corticosteroids in the TMJ. It may thus be considered as an advantage that the results are not influenced by the efficient general treatment effect of biologics, which can also affect the TMJ.

This project has a prospective cohort study design, and was approved by the regional ethical committee at Karolinska Institutet, Stockholm, Sweden (176/91; 310/97; 142/02; 03-2004) according to the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Assessment of subjective symptoms and clinical signs

The clinical examination included the assessment of several variables, further described below. All clinical examinations were performed by two experienced examiners (PA, SK), and the two examiners were calibrated regularly throughout the years, both theoretically and clinically, in order to prevent drift.

Variables on an individual level

The maximum voluntary mouth opening was measured in millimeters between the right central incisors, with the vertical overbite added.

The degree of anterior open bite (AOB) was assessed by recording of the occlusal contacts upon hard biting on a double occlusal foil in intercuspid position (2 x 8 µm, Occlusions-Prüf-Folie, GHM Hanel Medizinal, Nürtingen, Germany). Both the left side and the right side were assessed, and the following scores were used: 0 = occlusal contacts including the canine, 1 = no contacts anterior to the first premolar, 2 = no contacts anterior to the second premolar, 3 = no contacts anterior to the first molar, 4 = no contacts anterior to the second molar, and 5 = no occlusal contact. The sum of the scores of both sides was used in the analyses as an estimation of the degree of AOB. None of the patients in this study were edentulous, and the possible score thus ranged from 0 to 9. Score 9 (4+5) means that only one contact between two opposing posterior molars exists on one side.

Variables on a joint level

Local TMJ pain intensity at rest was assessed using either a 10-cm visual analogue scale (VAS; converted to a score of 0-10) or a numerical rating scale (NRS; 0-10) with the end points “no pain” (score 0) and “worst pain ever experienced” (score 10). Despite the use of two types of scales throughout the years of examinations, minimal influence on the results is expected due to the high correspondence between the two types of scales. [13]

Besides maximum voluntary mouth opening, participants were asked to perform maximum protrusion and maximum laterotrusion to both sides. Crepitus was recorded as present if crepitus was palpable or audible during at least one of these movements.

Probable clinical arthritis was defined according to Alstergren et al., [14] were “probable TMJ arthritis” is considered present if a joint has the combination of pain on maximum mouth opening and a contralateral laterotrusion of less than 8mm.

Blood sampling

Venous blood was collected at the start of the first visit and immediately before the clinical examination, to determine serum levels of rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serotonin, as well as plasma levels of interleukine-1β (IL-1β). Rheumatoid factor titers below 15 IE/mL and C-reactive protein levels below 10 mg/L were considered as zero values according to the standard procedures of the accredited laboratory at the Department of Clinical Chemistry at Karolinska University Hospital, Huddinge, Sweden.

Treatment and examination schedule

After the clinical examination at the first visit (T0), painful TMJs received an injection with glucocorticoid methylprednisolone (40 mg/mL) with lidocaine (10 mg/mL) added (Depo-Medrol cum lidocaine; Pfizer AB, Täby, Sweden). A volume of 0.7-0.8 mL was injected in the upper joint compartment of the TMJ. All injections were administered by a dentist with a specialization in orofacial pain and dysfunction, several years of experience in the use of TMJ corticosteroid injections and more than 2500 synovial fluid samplings (SK or PA), without the

use of imaging guidance. Participants also received individualized care with self-care instructions, including jaw exercises.

For all patients, the clinical examination was repeated (T1) after a median (interquartile range; IQR) interval of 3.1 (2.1-9.0) weeks. Based on the clinicians' decision on desired follow-up within the clinical care setting, for 21 patients the clinical examination was repeated again (T2) after a median (IQR) interval of 6.3 (4.3-17.9) weeks between T1 and T2.

Statistical analyses

Non-parametric statistics were used throughout the study due to the characteristics of most measured variables. For descriptive statistics, median values and 25th/75th percentiles are presented. Differences is maximum voluntary mouth opening, anterior open bite, and pain intensity between time points were tested with the Wilcoxon signed ranks test. To assess the correlation between clinical effect of the corticosteroid injections and systemic variables, the changes in maximum voluntary mouth opening and pain intensity between T0 and T1 were used as the clinical variables. The significance of correlations was tested with the Spearman's ranked correlation test. A probability level of $p<0.05$ was considered as significant.

Table 1. Characteristics of the study sample.

	Median	Percentile 25th	Percentile 75th	n
Age (y)	54	38	62	35
Gender (M/F)				4/31
Duration of general joint symptoms (y)	8	4	20	34
Time between onset of general and TMJ symptoms (y)	4	1	15	33
RF positivity, n (%)				22 (63)
Erythrocyte sedimentation rate	27	18	42	30
C-reactive protein	11	0.01	28	33
Thrombocyte particle count	335	274	420	32
Time between T0 and T1 (weeks)	3.1	2.1	9	35
Time between T1 and T2 (weeks)	6.3	4.3	17.9	21

N = number of observations, TMJ = temporomandibular joint, RF = rheumatoid factor

Results

Data were collected for 70 joints in 35 RA patients. Table 1 shows the characteristics of the study sample. During the first visit (T0), all patients received a corticosteroid injection in one or both joints; in total, 53 out of 70 joints were injected. All patients had a second visit (T1), and 21 patients also had a third visit (T2), out of whom 20 patients received another TMJ injection during the visit at T1.

Maximum mouth opening capacity

The median maximum mouth opening capacity significantly increased between T0 and T1 (from 37mm to 40mm, p=0.004). For the 21 patients that had a third visit, the median maximum mouth opening capacity slightly increased between T1 and T2, but this change was not significant (from 39mm to 40mm, p=0.139), and there also was no significant difference between T0 and T2 (p=0.432; Fig. 1).

Anterior open bite

The AOB did not differ significantly between T0 and T1 (median 0 and 1, respectively, p=0.307), and also not between T1 and T2 (median 1 and 0, respectively, p=0.109), nor between T0 and T2 (median 0 for both, p=0.478) for patients that had three visits.

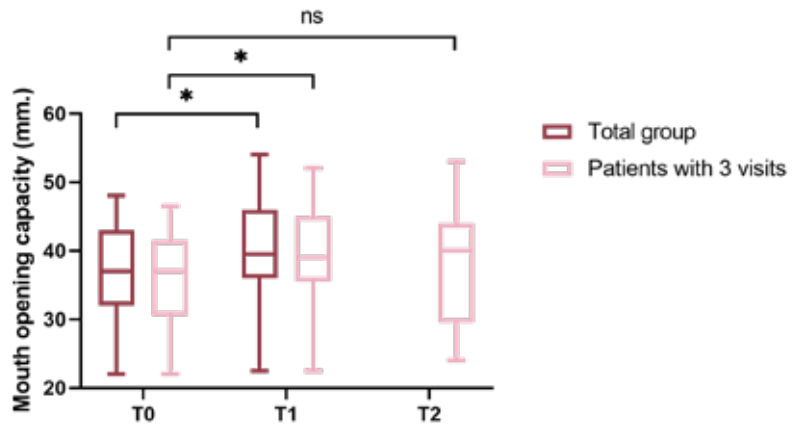


Figure 1. Maximum mouth opening capacity in 35 patients with rheumatoid arthritis. All patients received a corticosteroid injection in one or both temporomandibular joints (TMJs) at T0, of whom 21 patients had a third visit at T2. The median (IQR) interval was 3.1 (2.1-9.0) weeks between T0 and T1, and 6.3 (4.3-17.9) weeks between T1 and T2. A significant result ($p < 0.05$) is indicated by an asterisk, while 'ns' indicates no statistically significant difference.

Temporomandibular joint pain intensity

Results are presented in Figure 2. The TMJ-pain intensity at rest significantly decreased between T0 and T1 (median 3 and 2, respectively, p=0.001) for the joints that received an injection at T0. For patients that had a third visit, no further difference was found between T1 and T2 (median 2 and 4, respectively, p=0.123), nor between T0 and T2 (median 5 and 4, respectively, p=0.228). For the joints that did not receive an injection at T0, no differences in TMJ-pain intensity over time were found (median 0 at all time points, Fig. 2).

Crepitus

Data on crepitus were available for 68 joints. Figure 3 shows the transitions from crepitus to no crepitus and vice versa between T0 and T1, for 52 joints that received a corticosteroid injection at T0, and 16 joints that did not receive a corticosteroid injection at T0. At T2, data are available for 34 joints that received an injection at T0, of which eleven joints had crepitus – two new cases, and nine joints that already had crepitus at T0 and/or T1. Of the joints that did not receive an injection at T0, only one joint had crepitus at T1. This joint received a corticosteroid injection at T1 and was then crepitus-free at T2.

Clinical arthritis

Data on probable clinical arthritis is available for 64 joints. Figure 3 shows the transition from probable clinical arthritis to no clinical arthritis and vice versa between T0 and T1, for 47 joints that received a corticosteroid injection at T0, and 17 joints that did not receive a corticosteroid injection at T0.

Relation with pretreatment systemic variables

The change in maximum voluntary mouth opening between T0 and T1 did not correlate to pretreatment serum levels of ESR (r_s=-0.057, n=30, p=0.766), CRP (r_s=-0.221, n=33, p=0.215), and serotonin (r_s=-0.273, n=28, p=0.160), nor to plasma levels of IL-1β (r_s=-0.257, n=27, p=0.196).

The change in pain intensity between T0 and T1 also did not correlate to pretreatment serum levels of ESR (r_s=0.087, n=36, p=0.613), CRP (r_s=0.202, n=40, p=0.212), and serotonin (r_s=0.213, n=36, p=0.213), nor to plasma levels of IL-1β (r_s=-0.238, n=31, p=0.196).

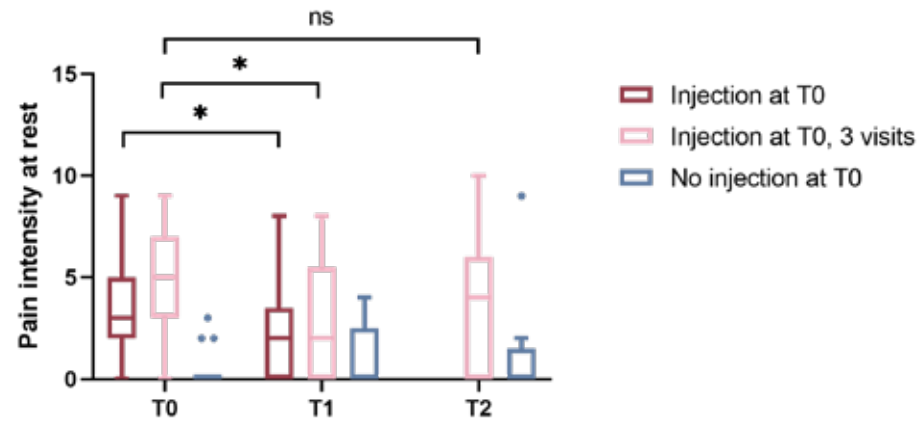


Figure 2. Pain intensity at rest in the temporomandibular joint (TMJ) of patients with rheumatoid arthritis. Forty-seven TMJs received a corticosteroid injection at T0, of which for 29 TMJs data is available during a third visit at T2, while 17 TMJs in the same patient group did not receive corticosteroid injections at T0. The median (IQR) interval was 3.1 (2.1-9.0) weeks between T0 and T1, and 6.3 (4.3-17.9) weeks between T1 and T2. A significant result ($p < 0.05$) is indicated by an asterisk, while 'ns' indicates no statistically significant difference.

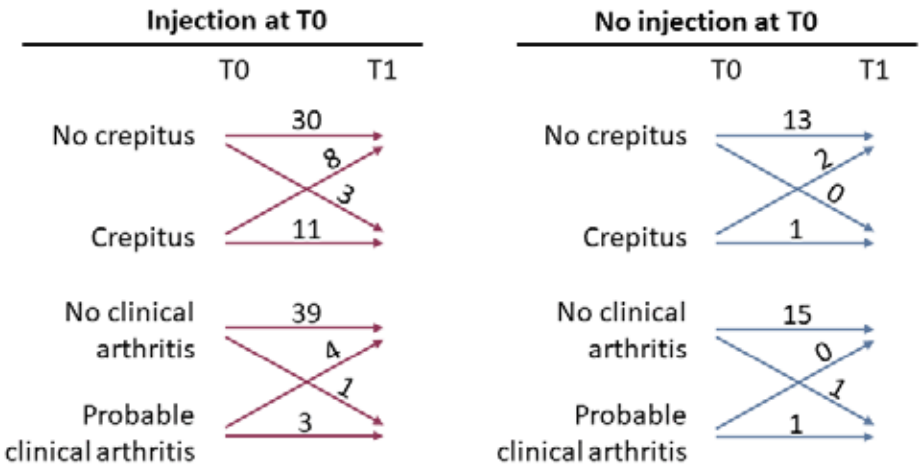


Figure 3. Crepitus and fulfillment of the clinical criteria for arthritis in the temporomandibular joint of patients with rheumatoid arthritis. Numbers of transitions between T0 and T1 from crepitus to no crepitus and vice versa (in 68 joints, of which 52 joints received a corticosteroid injection at T0), and from probable clinical arthritis to no clinical arthritis and vice versa (in 64 joints, of which 47 joints received a corticosteroid injection at T0). The median (IQR) interval was 3.1 (2.1-9.0) weeks between T0 and T1.

Discussion

This study indicates that methylprednisolone injections in a painful TMJ of patients with RA alleviate symptoms and improve function for approximately three weeks. Although all injected joints were painful, most joints did not fulfill the novel diagnostic criteria for "probable clinical TMJ arthritis". This study could not establish that pretreatment systemic inflammatory activity is related to the treatment effect.

The methylprednisolone used for TMJ injection in this study is a crystalized corticosteroid with small to medium sized crystals, that are known to retain in the tissues with pharmacological effects for one to three weeks after injection, [15] which corresponds to our results. However, while injection in the RA knee joint results in a duration of remission for approximately eight weeks, and decrease in joint effusion can even last up to one year, [7] the current results show that both pain and dysfunction in the TMJ revert to post-treatment levels relatively quickly after the injection. This corresponds to the short to medium term effects of intra-articular injection with corticosteroids found in other populations with temporomandibular dysfunction (TMD). [16] To attain long-term improvement of TMJ symptoms and function, addition of other treatment modalities is crucial. Despite the short duration, the combination of decrease in pain intensity and increase in maximum mouth opening achieved by corticosteroid injection does offer patients a temporary relief, but also allows them to perform jaw exercises without being hindered by pain. Injections could thus be used to facilitate additional noninvasive, conservative treatment of TMD.

As with any treatment, possible negative effects should always be considered when deciding on a treatment strategy, especially since corticosteroids have potent effects on most cell types. In case of corticosteroid injections in the hip and knee of osteo-arthritis (OA) patients, adverse joint findings such as accelerated OA progression have been observed. [17] However, a study on corticosteroid injections in several types of joints – both large and small – of RA patients, that included evaluation of possible negative effects, showed a good tolerance and no serious adverse events, suggesting that the positive results outweigh the possible negative consequences in this patient group. [18] Further, none of the studies that have investigated side effects such as accelerated bone tissue destruction have taken into account the intra-articular inflammatory activity. Since arthritis can cause pain as well as cartilage and bone tissue destruction, these effects may not be related to the treatment with corticosteroids. Given that, possible side effects like skin atrophy in case of subcutaneous deposition and transient pain due to crystal-induced synovitis, are well documented adverse effects of corticosteroid injections. To minimize the risk of negative consequences, it is prudent to have the injections administered by experienced clinicians, which was the case within this study. However, no imaging guidance was used during the procedure. Several studies demonstrate increased accuracy of the injection procedure with ultrasound guidance, [19] although efficacy of the ultrasound-guided injections seems similar to palpation-guided injections, as demonstrated in several joints of RA patients. [20] We thus expect no relevant influence of the lack of imaging guidance on the clinical results of this study.

In this study, the change in maximum mouth opening and pain intensity were not related to pretreatment systemic inflammatory activity. However, in a population of patients with various rheumatic diseases, Fredriksson et al. [11] found an association between treatment effect and pretreatment systemic levels of serotonin. Our finding is also in contrast with earlier findings of Alstergren et al. in seropositive RA patients, where TMJ pain on mandibular movement was correlated to systemic factors. [21] The current results do not confirm this association for RA patients, indicating that more research into how systemic inflammatory activity affects symptoms of TMJ arthritis is needed. In further research aimed at phenotyping patients that benefit from specific treatment modalities, preferably both systemic and local inflammatory mediators should be taken into account. The study by Fredriksson et al. shows that treatment effect of intra-articular corticosteroid injections was also associated with pretreatment levels of serotonin in local TMJ synovial fluid. [11] However, it was not possible to take this factor into account while analyzing the current results, because complete data on synovial fluid were not available.

No significant changes in the degree of anterior open bite (AOB) were found over time. AOB can be used as a coarse clinical sign of tissue destruction, and may develop over time in RA patients with TMJ involvement. [4] The duration of the current study was most likely too short to be able to observe a possible change in AOB, at least to be able to detect normalization due to a possible arrested bone tissue loss by the treatment and subsequent normalization of occlusion. A longer follow-up period would be necessary to investigate whether TMJ tissue destruction can be prevented by using corticosteroid injections. A study by Vallon et al. [22] with a 12-year follow-up did show a positive long-term result of TMJ corticosteroid injections in patients with RA on radiological signs of structural bone changes. However, the drop-out rate was high – only 12 out of

the original 41 participants were examined clinically and radiographically after 12 years – and systemic treatments were not taken into account. Their results must therefore be interpreted with caution.

The limitations of the current study that may influence the generalizability of the conclusions, further discussed below, include the lack of a control group, the lack of information on individualized care regarding the TMJ, the use of the 1987 classification criteria for RA, the lack of detailed information on systemic pharmacological treatment, and that the medication profiles presumably do not fully correspond to current guidelines.

In studies that measure the effect of a certain treatment, it is preferable to have a non-treatment control group. On the other hand, one of the main aims of this study was to relate treatment effects to systemic inflammatory activity. In the current study, 17 joints did not receive treatment with a corticosteroid injection and showed no change in pain intensity over time. However, these joints cannot be considered as pure controls because the decision to inject was based on baseline pain intensity. In these joints, pain intensity was very low to absent throughout the duration of the study, and they are thus difficult to compare to the joints that did receive an injection. In addition, anti-inflammatory treatment in one joint may influence the contralateral joint through systemic or central mechanisms.

In addition to the corticosteroid injections, participants within this study received individualized care with self-care instructions, including jaw exercises. However, specific information was not available to take into account during the analysis of the results. Further, the clinical relevance of the decrease in pain intensity and increase in maximum mouth opening capacity were not measured on a subjective level. In future research, the combination of corticosteroid injections and other treatment modalities, and the subjective effect of the injections – for example as measured by possible changes in oral health-related quality of life – deserve further attention.

Since patients were included between 1990 and 2006, RA was diagnosed according to the 1987 classification criteria of the American College of Rheumatology (ACR). In 2010, the ACR and the European League Against Rheumatism (EULAR) published revised classification criteria. [23] These 2010 ACR/EULAR criteria put more emphasis on RA characteristics that emerge early in the disease course, in order to identify and treat RA patients earlier. However, the aim of this study was to evaluate the effect of TMJ corticosteroid injections in RA patients in general, regardless of their general disease duration. With a median duration of general joint symptoms of 8 years, we do not expect a significant influence of the 1987 classification criteria on the selected patient group. It does mean that patients possibly received a different pharmacological treatment, and at a later stage than they would have nowadays, as further discussed below.

Specific data on the pharmacological treatment of patients were not available and could thus not be analyzed as a possible confounding factor. The medication profiles presumably do not fully correspond to current guidelines, since the period of data collection was mostly before the introduction of biologics, which means that results can only be generalized to RA patients that are not on biologic therapy. However, it may also be considered an advantage as previously mentioned. Furthermore, although developments in the treatment of RA have substantially changed the course of the disease, nowadays frequently resulting in remission, [24,25] recent studies still report a high prevalence of TMJ involvement in RA. [26,27] In patients with early RA, systemic pharmacological treatment seemed to have a positive effect on TMJ involvement for some, but not all patients. [28,29] This corresponds to the course of RA disease in general, where individual joints can display persistent complaints or temporary flare-ups, resulting in the continuous use of corticosteroid injections in clinical practice. An interesting development in research to monitor in this context is the focus on injection with anti-TNF agents as an alternative for glucocorticoids. [30,31] Altogether, this suggests that, despite the greatly improved treatment modalities, targeted treatment for the TMJ remains necessary for a number of RA patients.

Conclusion and implications

This study indicates that corticosteroid injections with methylprednisolone in the TMJ alleviate pain and improve mouth opening capacity for approximately three weeks. The temporary relief achieved with an injection can facilitate patients to perform jaw exercises, without being limited by pain. Corticosteroid injections thus seem useful for the short-term management of TMJ involvement in RA.

Acknowledgements

The authors would like to thank the subjects that participated and Mrs Karin Trollsås for invaluable support in our laboratory.

Funding

This study was financially supported by grants from the Swedish Medical Research Council (grant 10416); the Institute of Odontology, Karolinska Institutet, Huddinge, Sweden; the Swedish National Association against Rheumatism, Stockholm, Sweden; Signe and Reinhold Sund's Foundation, Stockholm, Sweden and the Swedish Dental Association, Stockholm, Sweden.

References

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376:1094-1108.
2. Kourilovitch M, Galarza-Maldonado C, Ortiz-Prado E. Diagnosis and classification of rheumatoid arthritis. J Autoimmun. 2014;48-49:26-30.
3. Savtekin G, Sehirli AO. Rheumatoid arthritis in temporo-mandibular joint: A review. Niger J Clin Pract. 2018;21:1243-1246.
4. Kroese JM, Kopp S, Lobbezoo F, Alstergren P. TMJ Pain and Crepitus Occur Early Whereas Dysfunction Develops Over Time in Rheumatoid Arthritis. J Oral Facial Pain Headache. 2020;34:398-405.
5. Dahlström L, Carlsson GE. Temporomandibular disorders and oral health-related quality of life. A systematic review. Acta Odontol Scand. 2010;68:80-85.
6. Smolen JS, Landewe RBM, Bijlsma JWJ, Burmester GR, Dougados M, Kerschbaumer A, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis. 2020;79:685-699.
7. Habib GS, Saliba W, Nashashibi M. Local effects of intra-articular corticosteroids. Clin Rheumatol. 2010;29:347-356.
8. Haggman-Henrikson B, Alstergren P, Davidson T, Hogestatt ED, Ostlund P, Tranaeus S, et al. Pharmacological treatment of oro-facial pain - health technology assessment including a systematic review with network meta-analysis. J Oral Rehabil. 2017;44:800-826.
9. Kopp S, Akerman S, Nilner M. Short-term effects of intra-articular sodium hyaluronate, glucocorticoid, and saline injections on rheumatoid arthritis of the temporomandibular joint. J Craniomandib Disord. 1991;5:231-238.
10. Fredriksson L, Alstergren P, Kopp S. Tumor necrosis factor-alpha in temporomandibular joint synovial fluid predicts treatment effects on pain by intra-articular glucocorticoid treatment. Mediators Inflamm. 2006;2006:59425.
11. Fredriksson L, Alstergren P, Kopp S. Serotonergic mechanisms influence the response to glucocorticoid treatment in TMJ arthritis. Mediators Inflamm. 2005;2005:194-201.
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31:315-324.
13. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, et al. Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review. J Pain Symptom Manage. 2011;41:1073-1093.
14. Alstergren P, Pigg M, Kopp S. Clinical diagnosis of temporomandibular joint arthritis. J Oral Rehabil. 2018;45:269-281.
15. Ritter JFR, Henderson G, Loke YK, MacEwan D, Rang H. Rang & Dale's Pharmacology. 2019. 9th edition. Elsevier.
16. Machado E, Bonotto D, Cunali PA. Intra-articular injections with corticosteroids and sodium hyaluronate for treating temporomandibular joint disorders: a systematic

review. Dental Press J Orthod. 2013;18:128-133.
17. Kompel AJ, Roemer FW, Murakami AM, Diaz LE, Crema MD, Guermazi A. Intra-articular Corticosteroid Injections in the Hip and Knee: Perhaps Not as Safe as We Thought? Radiology. 2019;293:656-663.
18. Hetland ML, Ostergaard M, Ejbjerg B, Jacobsen S, Stengaard-Pedersen K, Junker P, et al. Short- and long-term efficacy of intra-articular injections with betamethasone as part of a treat-to-target strategy in early rheumatoid arthritis: impact of joint area, repeated injections, MRI findings, anti-CCP, IgM-RF and CRP. Ann Rheum Dis. 2012;71:851-856.
19. Kane D, Koski J. Musculoskeletal interventional procedures: With or without imaging guidance? Best Pract Res Clin Rheumatol. 2016;30:736-750.
20. Nordberg LB, Lillegraven S, Aga AB, Sexton J, Lie E, Hammer HB, et al. The Impact of Ultrasound on the Use and Efficacy of Intraarticular Glucocorticoid Injections in Early Rheumatoid Arthritis: Secondary Analyses From a Randomized Trial Examining the Benefit of Ultrasound in a Clinical Tight Control Regimen. Arthritis Rheumatol. 2018;70:1192-1199.
21. Alstergren P, Fredriksson L, Kopp S. Temporomandibular joint pressure pain threshold is systemically modulated in rheumatoid arthritis. J Orofac Pain. 2008;22:231-238.
22. Vallon D, Akerman S, Nilner M, Petersson A. Long-term follow-up of intra-articular injections into the temporomandibular joint in patients with rheumatoid arthritis. Swed Dent J. 2002;26:149-158.
23. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-1588.
24. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet. 2007;370:1861-1874.
25. Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017;389:2338-2348.
26. Kurtoglu C, Kurkcu M, Sertdemir Y, Ozbek S, Gurbuz CC. Temporomandibular disorders in patients with rheumatoid arthritis: A clinical study. Niger J Clin Pract. 2016;19:715-720.
27. González-Chávez SA, Pacheco-Tena C, Campos Torres RM, Quiñonez-Flores CM, Reyes-Cordero G, Caraveo Frescas TJ. Temporomandibular and Odontological Abnormalities in Patients with Rheumatoid Arthritis. Reumatol Clin. 2020;16:262-271.
28. Chin Jen Sem JP, van der Leeden M, Visscher CM, Britsemmer K, Turk SA, Dekker J, et al. Prevalence, Course, and Associated Factors of Pain in the Temporomandibular Joint in Early Rheumatoid Arthritis: Results of a Longitudinal Cohort Study. J Oral Facial Pain Headache. 2017;31:233-239.
29. Kroese JM, Volgenant CMC, Crielaard W, Loos B, van Schaardenburg D, Visscher CM, et al. Temporomandibular disorders in patients with early rheumatoid arthritis and at-risk individuals in the Dutch population: a cross-sectional study. RMD Open. 2021;7(1):e001485.
30. Carubbi F, Zugaro L, Cipriani P, Conchiglia A, Gregori L, Danniballe C, et al. Safety and efficacy of intra-articular anti-tumor necrosis factor alpha agents compared to corticosteroids in a treat-to-target strategy in patients with inflammatory arthritis and monoarthritis flare. Int J Immunopathol Pharmacol. 2016;29:252-266.
31. Salem RM, El-Deeb AE, Elsergany M, Elsaadany H, El-Khouly R. Intra-articular injection of etanercept versus glucocorticoids in rheumatoid arthritis patients. Clin Rheumatol. 2021;40:557-564.

The oral cavity

Differences in the oral microbiome in patients with early rheumatoid arthritis and individuals at risk of rheumatoid arthritis compared to healthy individuals

Johanna M. Kroese, Bernd W. Brand, Marc J. Buijs, Wim Crielaard, Frank Lobbezoo, Bruno G. Loos, Laurette van Boheemen, Dirkjan van Schaardenburg, Egija Zaura, Catherine M.C. Volgenant

Published in: Arthritis & Rheumatology. 2021;73(11):1986-1993.
PMID: 33949151.

Chapter 06

ABSTRACT

Objective: It has been suggested that rheumatoid arthritis (RA) may originate at the oral mucosa. Our aim was to assess the oral microbiome and the periodontal condition in patients with early rheumatoid arthritis (ERA) and individuals at risk of RA.

Methods: Three groups were recruited (50 participants each): (1) ERA patients (2010 ACR/EULAR criteria), (2) at-risk individuals (arthralgia and autoantibodies), and (3) healthy controls. A periodontal examination resulted in scores for bleeding on probing (BOP), pocket probing depth (PPD), and periodontal inflamed surface area (PISA). The microbial composition of subgingival dental plaque, saliva, and tongue coating was assessed using 16S rDNA amplicon sequencing, and compared between groups with permutational multivariate analyses of variance (PERMANOVA).

Results: There was no difference between the groups on the periodontal variables (BOP p=0.70; PPD p=0.30; PISA p=0.57). PERMANOVA showed a difference between the groups in the microbial composition of saliva (F=2.08, p<0.001) and tongue coating (F=2.04, p=0.008), but not plaque (p=0.51). Post-hoc tests showed no difference between the ERA group and at-risk group (saliva F=1.12, p=0.28; tongue coating F=0.834, p=0.59). Discriminative zero-radius operational taxonomic units (zOTUs) were identified: in ERA patients and at-risk individuals, *Prevotella* in saliva and *Veillonella* in saliva and tongue coating were at higher relative abundance compared to healthy controls.

Conclusion: The results show similarities in the oral microbiome between ERA patients and at-risk individuals, both presenting with increased relative abundance of potentially pro-inflammatory species compared to healthy controls, suggesting a possible association between the oral microbiome and RA onset.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, frequently accompanied by autoantibodies such as rheumatoid factor (RF) and antibodies against citrullinated proteins (ACPA). [1] These antibodies are often present several years before the onset of clinically apparent RA. [2]

It has been suggested that RA originates at mucosal sites, such as the gut and oral mucosa. [3,4] Periodontitis – a chronic inflammation of the gingiva, tooth-supporting connective tissues and the alveolar bone – displays pathogenic similarities to RA, and several studies show an association between periodontal disease and RA. [5] In previous studies on the oral microbiome in relation to RA, there was a particular interest in *Porphyromonas gingivalis* (Pg), a bacterium associated with periodontal disease. [6] Because of its capacity to generate citrullinated proteins, Pg could potentially trigger ACPA production and thereby initiate an RA-associated immune response. [1]

However, previous studies in both RA patients and individuals at risk of RA suggest that further studies should focus on the microbiome as a whole rather than on specific species. [7-9] Since the oral microbiome may play a role in RA onset, and may thus be a target for RA prediction or even prevention, information on patients with early RA (ERA) and individuals at risk of RA is most relevant. However, data on these specific groups is currently very limited. Our aim was therefore to assess the oral microbiome and the periodontal condition in patients with ERA and individuals at risk of RA, compared to each other and compared to healthy controls.

Materials and methods

Study design and ethical approval

This study is based on baseline data of a larger parent cohort-study, and a full description of the protocol has been published. [10] Below, an outline of the protocol is given. The information on sample processing (appendix) is a pertinent addition to the earlier published paper. The protocol was approved by the accredited Medical Ethical Committee of the Slotervaart Hospital and Reade (METc Slotervaartziekenhuis and Reade, U/17.056/P1719), and is included in the Dutch National Trial Register (NTR, NTR6362; https://www.trialregister.nl/trial/6198).

Participants and recruitment

In brief, three groups of participants were recruited: (1) patients with ERA, (2) individuals at risk of RA, and (3) a control group without auto-immune conditions. Groups 1 and 2 were recruited at Reade, a rheumatology clinic in Amsterdam, The Netherlands. Group 1 consisted of patients diagnosed with RA within the previous year and fulfilling the 2010 ACR/EULAR RA criteria. [11] For group 2, participants were recruited from the Reade at-risk cohort. [12-14] Participants in this cohort have inflammatory-type arthralgia combined with increased serum levels of RF and/or ACPA. Participants for group 3 were recruited at the Academic Centre for Dentistry Amsterdam (ACTA), unselective for oral status, and were matched to groups 1 and 2 for sex and age (±5 years). All participants were ≥18 years, had a minimum of 12 natural teeth, and gave written informed consent. All clinical examinations and sampling took place at Reade and were performed by a single trained dentist (JMK).

Outcome variables

General health

All participants completed a medical questionnaire prior to the research visit, to identify possible confounders like comorbid conditions. During the research visit additional questions were asked about antibiotics use during the past three months. Venous blood was collected to determine serum levels of RF and ACPA. Blood samples were processed by the haematological laboratory of the OLVG Hospital in Amsterdam, using the Phadia 250 EliA IgM and Phadia 250 EliA CCP tests, respectively (Phadia AB, Uppsala, Sweden). According to the manufacturer's standards, individuals with RF levels >5.0 kU/l and/or ACPA levels >10.0 kU/l were considered seropositive; otherwise, participants were considered seronegative.

Oral health

Participants were asked about the time since brushing their teeth and regular practice of additional oral hygiene measures, e.g., mouth rinse and tongue cleaning. An intra-oral examination was performed to determine the total number of teeth present, the number of decayed, missing, and filled teeth (DMFT), and the presence or absence of a removable (partial) denture.

Collection of samples for microbiome analyses

Participants were instructed not to perform any oral hygiene measures for 24 hours, and not to eat or drink anything except water for two hours prior to their research visit. During the visit, a subgingival dental plaque, saliva and tongue coating sample were collected. The microbial composition of the samples was assessed using 16S rDNA amplicon sequencing. A thorough description of sample collection and processing is available in the appendix.

Periodontal examination

The periodontal examination included registration of bleeding on probing (BOP, absent/present) and pocket probing depth (PPD) in millimeters on six sites for each tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual). The dentist performing the examination (JMK) was trained by a periodontist.

Based on the PPD registrations, the Community Periodontal Index of Treatment Need (CPITN) score [15] was calculated, and used to categorize participants according to periodontitis status. Participants were categorized as either suspected for severe periodontitis (CPITN score 4) or not (CPITN score 0-3), which means that a PPD of ≥6mm for at least one tooth was needed to be categorized as being suspected for severe periodontitis.

Recording of BOP resulted in a full-mouth BOP percentage. When calculating this percentage, data were not used from the tooth of which a subgingival plaque sample was collected. The total periodontal inflamed surface area (PISA) was calculated using the method described by Nesse et al., [16] to quantify the total burden of periodontal inflammation.

Data analysis

Descriptive statistics were used to describe the characteristics of the study population. For continuous variables, a one-way ANOVA was performed when comparing the means of the three groups on normally distributed variables; otherwise, a Kruskal-Wallis test was used. Possible differences between groups on categorical variables were tested with a Chi-square test. A two-sided alpha level of 0.05 was used for the statistical analyses.

Microbiome data among the three groups was analysed on composition and microbial diversity for all three niches, i.e., plaque, saliva, and tongue coating. Details on these analyses are available in the appendix.

Additionally, since ACPA is a more relevant predictor for RA than RF, [17] within the at-risk group two subgroups – ACPA positive versus ACPA negative – were compared on possible confounding factors and periodontal variables, and microbiome composition for all three niches.

Results

Characteristics of the study population

From November 2017 until July 2019, 150 participants were included, 50 per group (Table 1). In the ERA group, patients were included on average 3.1±1.7 months after being diagnosed with RA. The majority of ERA patients was treated with methotrexate, mostly in combination with prednisone, according to the national guideline on drug treatment of RA [18] (Table 1).

The three groups were compared on factors that could influence the oral microbiome: smoking, alcohol consumption, use of drugs, use of painkillers during the past 24 hours, use of antibiotics during the past three months, wearing removable dentures, regular tongue cleaning, regular use of mouth rinse, DMFT, time since eating/drinking, and time since oral hygiene (appendix Table 1). The groups did not differ on any but one of the aforementioned variables: patients in the ERA group performed oral hygiene statistically significantly shorter before the research visit compared to the other two groups.

Table 1. Characteristics of the study population.[1]

	ERA group (n=50)	At-risk group (n=50)	Control group (n=50)	*p*
Age, years [mean (SD)]	52.1 (13.2)	51.4 (10.3)	51.2 (11.0)	0.92[a]
Gender, female [n (%)]	39 (78%)	38 (76%)	38 (76%)	0.96[b]
RF positive [n (%)]	37 (74%)	46 (92%)	0 (0%)	-[c]
ACPA positive [n (%)]	31 (62%)	24 (48%)	0 (0%)	-[c]
RF and/or ACPA positive [n (%)]	38 (76%)	50 (100%)	0 (0%)	-[c]
Pharmacological treatment for RA				
Methotrexate [n (%)]	44 (88%)	-	-	-
Prednisone [n (%)]	39 (78%)	-	-	-
Other [n (%)]	4 (8%)	-	-	-
No pharmacological treatment [n (%)]	2 (4%)	-	-	-

n = number of observations, ERA = early rheumatoid arthritis, RF = rheumatoid factor, ACPA = anti-citrullinated protein antibodies

[1] Extensive data are available in appendix Table 1.

[a] One-way ANOVA, [b] Chi-square test, [c] A difference in RF or ACPA positivity was not tested between the groups, because seropositivity was an inclusion criterion for the at-risk group and an exclusion criterion for the control group, and a difference is thus obvious.

Periodontal health

There was no difference in BOP percentage, average PPD, or PISA between the three groups (Table 2). Although a trend was seen towards a higher prevalence of severe periodontitis in the ERA group and at-risk group compared to the control group, with an increase in prevalence from the control group to the at-risk group, and from the at-risk group to the ERA group, no significant difference among the groups was found (Table 2).

ACPA positive versus ACPA negative at-risk individuals

A separate analysis was performed to compare ACPA positive (n=24) and ACPA negative (n=26) at-risk individuals on all aforementioned possible confounding factors and periodontal variables. No differences were found between these two subgroups (data not shown).

Table 2. Bleeding on probing (BOP), pocket probing depth (PPD), and periodontal inflamed surface area (PISA) in the study sample.

	ERA group (n=50)	At-risk group (n=50)	Control group (n=50)	*p*
BOP (%) [median (IQR)]	19.3 (9.9-35.4)	15.4 (7.4-32.5)	17.5 (8.5-27.5)	0.70[a]
Average PPD (six sites) of plaque sample tooth, mm. [median (IQR)]	2.5 (2.3-3.0)	2.6 (2.3-3.0)	2.5 (2.2-2.8)	0.53[a]
Average PPD, mm [median (IQR)]	2.2 (2.0-2.6)	2.2 (2.0-2.5)	2.1 (1.9-2.5)	0.30[a]
Number of pockets ≥6mm [median (IQR)]	0 (0-0.25)	0 (0-0)	0 (0-0)	0.33[a]
PISA, mm2 [median (IQR)]	258.3 (108.4-398.3)	191.3 (72.5-431.8)	181.4 (91.2-369.4)	0.57[a]
CPITN 4 [n (%)]	12 (24%)	10 (20%)	7 (14%)	0.21[b]

n = number of observations, ERA = early rheumatoid arthritis, BOP = bleeding on probing, PPD = pocket probing depth, PISA = periodontal inflamed surface area, CPITN = Community Periodontal Index of Treatment Needs

[a] Kruskal-Wallis, [b] Chi-square test, Linear-by-linear Association

Microbiological signatures

The samples had a total of 948 zero-radius operational taxonomic units (zOTUs) after processing. After subsampling at 3,500 reads per sample, 942 zOTUs remained with an average of 130 zOTUs per sample. Eight samples (two plaque, four saliva, and two tongue samples) were excluded from further analyses because the number of reads was too low. Because patients in the ERA group performed oral hygiene significantly shorter before the research visit compared to the other groups, and brushing could influence the oral microbiome, this variable was taken into account when analyzing group differences by permutational multivariate analyses of variance (PERMANOVA). Results on sample diversity – Shannon diversity index, number of zOTUs per sample, and Bray-Curtis distances – for all three niches are available in the appendix.

Plaque samples

There was no difference in microbial composition of plaque among the groups (two-way PERMANOVA based on hours since oral hygiene (F=1.58, p=0.070) and group (F=0.948, p=0.51)). The principal component analysis (PCA) did not show clustering by group either (Figure 1-A).

Saliva samples

The microbial composition of saliva differed significantly among the groups, irrespective of hours since oral hygiene (two-way PERMANOVA based on hours since oral hygiene (F=2.27, p=0.004, and group (F=2.08, p=0.0002)). The samples from the control group clustered together compared to the other two groups (Figure 1-B). This was confirmed by the post-hoc pairwise two-way PERMANOVA based on hours since oral hygiene and group, with a significant difference between the control group and ERA group (F=2.66, p<0.001) and the control group and at-risk group (F=2.56, p=0.001), but not between the ERA group and at-risk group (F=1.12, p=0.28).

Tongue coating samples

The microbial composition of tongue coating was also significantly different among the groups, irrespective of hours since oral hygiene (two-way PERMANOVA based on hours since oral hygiene (F=1.97, p=0.033) and group (F=2.04, p=0.008)). Again, the samples from the control group clustered together (Figure 1-C). This was confirmed by the post-hoc pairwise two-way PERMANOVA based on hours since oral hygiene and group, with a significant difference between the control group and ERA group (F=2.75, p=0.005) and the control group and at-risk group (F=2.59, p=0.01), but not between the ERA group and at-risk group (F=0.834, p=0.59).

ACPA positive versus ACPA negative at-risk individuals

For all three niches, a separate one-way PERMANOVA was performed to compare ACPA positive and ACPA negative at-risk individuals. No differences were found between these two subgroups (data not shown).

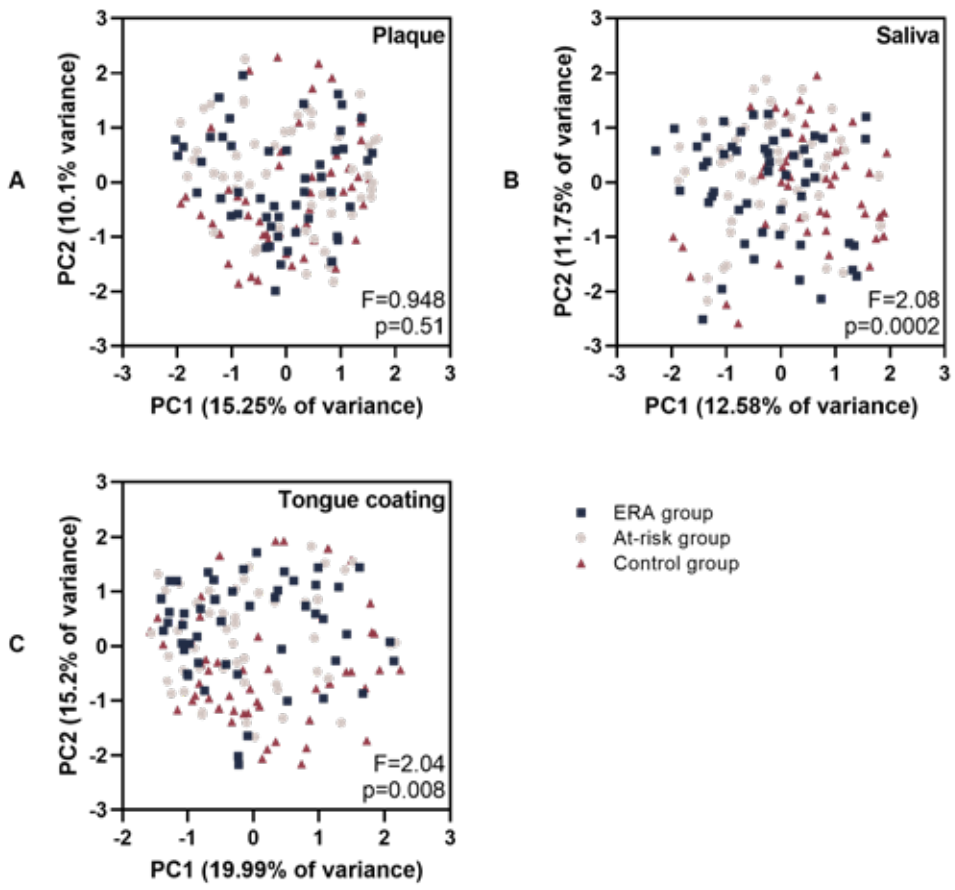


Figure 1. Principal component analysis (PCA) plots displaying PC1 (x-axis) and PC2 (y-axis) of the microbiological signatures of patients with early rheumatoid arthritis (ERA), individuals at risk of RA, and healthy controls in (A) subgingival dental plaque, (B) saliva, and (C) tongue coating. The F and p values indicate the results of two-way PERMANOVA analyses on hours since oral hygiene and group.

Discriminative zOTUs

Saliva samples

Twenty-five zOTUs significantly discriminated among the groups, of which seven with a relative abundance of ≥0.01 in at least 1 group (appendix Table 3). Post-hoc Mann-Whitney U tests showed that the significant results were predominantly caused by the difference of the ERA group and the at-risk group from the control group, rather than by the difference between the ERA group and the at-risk group (appendix Table 3). *Prevotella salivae (zOTU 25)*, *Veillonella (zOTU 4),* and *Prevotella (zOTU 10)* were more abundant in the ERA group and at-risk group compared to the control group (Figure 2 A-C), while *Neisseria flavescens / subflava (zOTU 7)*, *Porphyromonas pasteri / sp._oral_taxon_278 (zOTU 15),* and *Veillonella parvula (zOTU 12)* were more abundant in the control group compared to the other two groups. Only *Fusobacterium periodonticum (zOTU 13)* was more abundant in the at-risk group and the control group compared to the ERA group, while no difference was found between the at-risk group and the control group.

Tongue coating samples

Nineteen zOTUs significantly discriminated among the groups, of which four with a relative abundance of ≥0.01 in at least 1 group (appendix Table 4). Again, the significant results were predominantly caused by the difference of the ERA group and the at-risk group from the control group, rather than by the difference between the ERA group and at-risk group (appendix Table 4). While *Veillonella (zOTU 4)* was more abundant in the ERA group and at-risk group (Figure 2-D), *Neisseria flavescens / subflava (zOTU 7)* and *Streptococcus dentisani / infantis / mitis / oralis / sp._oral_taxon_058 (zOTU 1)* were more abundant in the control group compared to the other two groups. *Fusobacterium periodonticum (zOTU 13)* was more abundant in the control group compared to the ERA group, while no differences were found for the at-risk group compared to both other groups.

Porphyromonas gingivalis

Pg was not identified as a discriminative zOTU. One zOTU – *Porphyromonas gingivalis (zOTU 116)* – did classify as Pg, but had overall low abundance in all three niches and did not differ among the groups (plaque: median relative abundance of 0 in all groups, Kruskall-Wallis p=0.37; saliva: median relative abundance of 0 in all groups, Kruskall-Wallis p=0.47; tongue coating: median relative abundance of 0 in all groups, Kruskall-Wallis p=0.12).

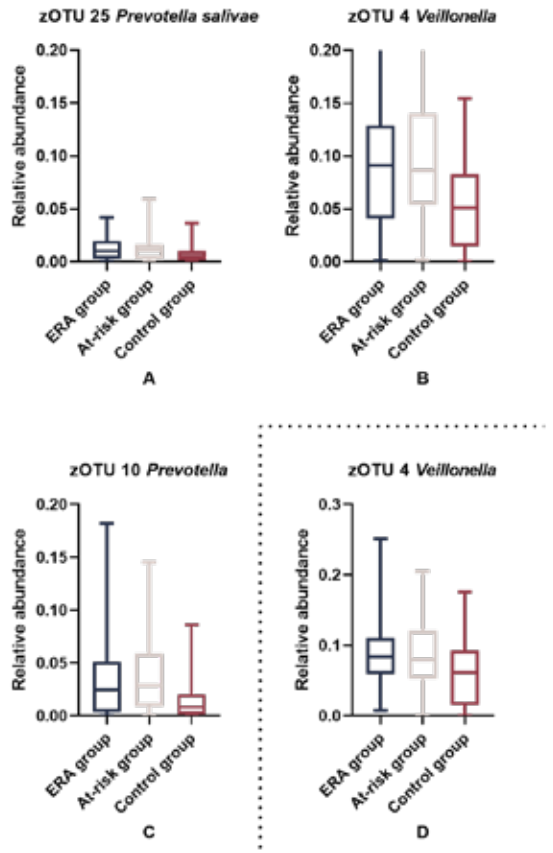


Figure 2. The relative abundance of the zero-radius operational taxonomic units (zOTUs) in (A, B, C) saliva and (D) tongue coating that discriminated among the groups according to the linear discriminant analysis (LDA) effect size (LEfSe) analysis, and were of a higher relative abundance in patients with early rheumatoid arthritis (ERA) and individuals at risk for RA compared to a healthy control group.

Discussion

Microbial composition of stimulated saliva and tongue coating, but not that of subgingival dental plaque, in patients with early rheumatoid arthritis (ERA) and individuals at risk of RA significantly differed from an age and sex matched healthy control group. *Prevotella* and *Veillonella* – both gram-negative anaerobes – were at higher relative abundance in saliva, and *Veillonella* was also at higher

relative abundance in tongue coating, of both ERA patients and at-risk individuals compared to healthy controls. However, in saliva there was also another zOTU that classified as a different species belonging to genus *Veillonella*, which was at higher relative abundance in the control group compared to the other two groups.

The increased relative abundance of genus *Prevotella* in ERA patients corresponds to the findings of Scher et al. in patients with new-onset RA, [7] and Correa et al. in patients with established RA. [19] Furthermore, studies on the gut microbiome also show an increased relative abundance of *Prevotella* species in ERA [3] and at-risk individuals. [20] Some *Prevotella* strains are capable of promoting chronic inflammation, by stimulating local cytokine production and induction of mucosal inflammation, which in turn can lead to systemic dissemination of inflammatory mediators. [21] Furthermore, a possible translocation of oral *Prevotella* species or their DNA to joint tissues in RA patients has been suggested. [22] Increased relative abundance of this potentially pro-inflammatory genus in ERA patients and at-risk individuals suggests a link between the oral microbiome and RA, [8] and microbiome dysbiosis may contribute to the induction of arthritis. Additionally, dysbiosis in both the oral and gut microbiome is partially resolved after the start of pharmacological RA treatment, [23] further supporting an association. However, the currently available literature is insufficient to establish a causal link and to fully comprehend the biological mechanism. [22] Furthermore, also a reversed pathway needs to be considered; due to active inflammation or based on the inflammation prone subject characteristics, the *Prevotella* species may find ecological advantages and emerge at larger numbers. When RA was treated with medication, [23] the ecology may revert to a situation less favorable for *Prevotella*. The possible influence of systemic RA treatment on the oral microbiome is overall a factor to consider when interpreting results of the ERA group. [24]

Due to the early stage of the disease, most ERA patients did receive the same treatment, and thus heterogeneity within the ERA group was prevented. However, it does mean that there was a notable difference with the at-risk group and the control group, causing a potential bias on the results. By including the ERA patients during the first few months after the start of the treatment, we attempted to limit this bias. Interestingly, the results still show an increased relative abundance of *Prevotella* in the ERA group and similarities to the at-risk group, possibly indicating a limited influence of the pharmacological treatment. However, it should be mentioned that future research should preferably include ERA patients without any immunomodulatory therapy.

This study is the first to report on the microbial composition of several oral niches in individuals at risk of RA. A study of Mankia et al. on ACPA-positive at-risk individuals reported on *Porphyromonas gingivalis* (Pg) and *Aggregatibacter Actinomycetemcomintans* (Aa) in subgingival plaque only, and found an increased relative abundance of Pg in at-risk individuals compared to healthy controls. [25] However, the current study showed an overall low abundance of Pg, and did not identify Pg as a relevant bacterium for discrimination between the groups. Also, we did not find differences between the ACPA positive and negative at-risk groups. Interestingly, the results do show an increased relative abundance of the *Veillonella* and *Prevotella* species in the at-risk group, showing resemblance to the findings in the ERA group. This corresponds to the findings of Tong et al., where both RA patients and at-risk individuals showed increased abundance of *Prevotella* in saliva. [26]

Furthermore, for most discriminative zOTUs, the relative abundance did not significantly differ between the ERA group and at-risk group. This corresponds to the overall similarities that were found in the microbiome between patients with ERA and at-risk individuals, despite the variety within these groups that should always be considered when interpreting Bray-Curtis distances between groups. Together, this indicates a possible role for oral microbial dysbiosis in the induction of arthritis, similar to findings on the gut microbiome. [3] It also corresponds to the findings of Cheng et al., where at-risk individuals, ERA patients, and controls showed differences in bacterial diversity and composition, and a role for oral microbiome dysbiosis in RA onset was suggested. [9] Collectively, the current findings and previous published data suggest that bacterial colonization on any mucosal tissues might trigger aberrant inflammatory reactions; this is not per se restricted to bacteria from periodontal pockets. However, the possible reversed pathway mentioned earlier cannot be excluded due to the cross-sectional nature of the data.

Regarding periodontal health and possible confounding factors, this study did not find differences between the groups. This is a major strength of the current study, since clinical differences between groups often is an issue that complicates the interpretation of results in microbiome studies. [27] It is, however, in contrast with the study of Mankia et al., where ACPA-positive at-risk individuals had a higher prevalence of periodontal disease compared to the control group. [25] This contrast might be explained by the difference in case definition of periodontitis. Further, the percentage of severe periodontitis in the healthy controls of the current study represents the prevalence in western populations, [28] while in the study of Mankia et al. prevalence of periodontitis was overall high, but severe cases were not specified. [25] Nonetheless, although not

significant, the current results do show a trend towards a higher prevalence of severe periodontitis in ERA and at-risk individuals compared to healthy controls.

A somewhat surprising result of this study is the absence of a difference in microbial composition of subgingival dental plaque among the groups. While previously a role for periodontal disease and associated bacteria in the onset of RA was hypothesized, [25] which would lead to a profound difference in plaque microbiome between the groups, the current results do not support this hypothesis. A possible explanation is the absence of a significant difference in periodontal disease among the groups, and thus no difference in relative abundance of associated bacteria. Further, the composition of dental plaque can be influenced by several factors, e.g., local immunological reactions, diet, and oral hygiene, while tongue coating was shown to be the most stable oral niche in microbiome composition. [29] Although we attempted to limit the influence of systemic treatment for RA as described earlier, it may also partly explain similarities in plaque microbiome between the ERA group and the control group, because the use of prednisone was shown to be associated with a healthier subgingival microbiome in patients with RA. [24] This effect may be more pronounced in subgingival plaque compared to saliva and tongue coating due to the direct contact of the subgingival niche to the circulatory system. However, further research is necessary to support this hypothesis.

To complement the currently available cross-sectional data, future studies should also include a longitudinal aspect, preferably with large cohorts and consistent data collection to aid the application of advanced methods, using artificial intelligence, for oral-systemic link prediction. [27] The current study does show similarities in the oral microbiome between ERA patients and at-risk individuals, both presenting with increased relative abundance of potentially pro-inflammatory species compared to healthy controls, and thus points toward a possible role for the oral microbiome in RA onset.

Acknowledgements

The authors would like to thank Mrs. Elly C. van Deutekom-Mulder for performing the DNA isolation of the microbiological samples.

References

1. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nat Rev Immunol. 2017;17:60-75.
2. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004;50:380-386.
3. Maeda Y, Takeda K. Host-microbiota interactions in rheumatoid arthritis. Exp Mol Med. 2019;51:1-6.
4. Konig MF. The microbiome in autoimmune rheumatic disease. Best Pract Res Clin Rheumatol. 2020;34:101473.
5. Cheng Z, Meade J, Mankia K, Emery P, Devine DA. Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2017;31:19-30.
6. Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, et al. Porphyromonas gingivalis: major periodontopathic pathogen overview. J Immunol Res. 2014;2014:476068.
7. Scher JU, Ubeda C, Equinda M, Khanin R, Buischi Y, Viale A, et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. Arthritis Rheum. 2012;64:3083-3094.
8. Lopez-Oliva I, Paropkari AD, Saraswat S, Serban S, Yonel Z, Sharma P, et al. Dysbiotic Subgingival Microbial Communities in Periodontally Healthy Patients With Rheumatoid Arthritis. Arthritis Rheumatol. 2018;70:1008-1013.
9. Cheng Z, Do T, Mankia K, Meade J, Hunt L, Clerehugh V, et al. Dysbiosis in the oral microbiomes of anti-CCP positive individuals at risk of developing rheumatoid arthritis. Ann Rheum Dis. 2021;80:162-168.
10. Kroese JM, Volgenant CMC, van Schaardenburg D, Loos BG, Crielaard W, Lobbezoo F. Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol. BDJ Open. 2020;6:7.
11. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-1588.
12. van Beers-Tas MH, Marotta A, Boers M, Maksymowych WP, van Schaardenburg D. A prospective cohort study of 14-3-3eta in ACPA and/or RF-positive patients with arthralgia. Arthritis Res Ther. 2016;18:76.
13. van Beers-Tas MH, Blanken AB, Nielen MMJ, Turkstra F, van der Laken CJ, Meursinge Reynders M, et al. The value of joint ultrasonography in predicting arthritis in seropositive patients with arthralgia: a prospective cohort study. Arthritis Res Ther. 2018;20:279.
14. Holla JFM, van Beers-Tas MH, van de Stadt LA, Landewe R, Twisk JWR, Dekker J, et al. Depressive mood and low social support are not associated with arthritis development in patients with seropositive arthralgia, although they predict increased musculoskeletal symptoms. RMD Open. 2018;4:e000653.

15. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J. Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J. 1982;32:281-291.
16. Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. J Clin Periodontol. 2008;35:668-673.
17. Bos WH, Wolbink GJ, Boers M, Tijhuis GJ, de Vries N, van der Horst-Bruinsma IE, et al. Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated protein antibody status: a prospective cohort study. Ann Rheum Dis. 2010;69:490-494.
18. Nederlandse Vereniging voor Reumatologie. Richtlijn Medicamenteuze Behandeling van Reumatoïde artritis. 2020. Available from: https://richtlijnendatabase.nl/richtlijn/reumato_de_artritis_ra/startpagina_-_reumatoide_artritis.html.
19. Correa JD, Fernandes GR, Calderaro DC, Mendonca SMS, Silva JM, Albiero ML, et al. Oral microbial dysbiosis linked to worsened periodontal condition in rheumatoid arthritis patients. Sci Rep. 2019;9:8379.
20. Alpizar-Rodriguez D, Lesker TR, Gronow A, Gilbert B, Raemy E, Lamacchia C, et al. Prevotella copri in individuals at risk for rheumatoid arthritis. Ann Rheum Dis. 2019;78:590-593.
21. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology. 2017;151:363-374.
22. Iljazovic A, Amend L, Galvez EJC, de Oliveira R, Strowig T. Modulation of inflammatory responses by gastrointestinal Prevotella spp. - From associations to functional studies. Int J Med Microbiol. 2021;311:151472.
23. Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med. 2015;21:895-905.
24. Beyer K, Zaura E, Brandt BW, Buijs MJ, Brun JG, Crielaard W, et al. Subgingival microbiome of rheumatoid arthritis patients in relation to their disease status and periodontal health. PLoS One. 2018;13:e0202278.
25. Mankia K, Cheng Z, Do T, Hunt L, Meade J, Kang J, et al. Prevalence of Periodontal Disease and Periodontopathic Bacteria in Anti-Cyclic Citrullinated Protein Antibody-Positive At-Risk Adults Without Arthritis. JAMA Netw Open. 2019;2:e195394.
26. Tong Y, Zheng L, Qing P, Zhao H, Li Y, Su L, et al. Oral Microbiota Perturbations Are Linked to High Risk for Rheumatoid Arthritis. Front Cell Infect Microbiol. 2019;9:475.
27. Seneviratne CJ, Balan P, Suriyanarayanan T, Lakshmanan M, Lee DY, Rho M, et al. Oral microbiome-systemic link studies: perspectives on current limitations and future artificial intelligence-based approaches. Crit Rev Microbiol. 2020;46:288-299.
28. Albandar JM. Underestimation of periodontitis in NHANES surveys. J Periodontol. 2011;82:337-341.
29. Zaura E, Pappalardo VY, Buijs MJ, Volgenant CMC, Brandt BW. Optimizing the quality of clinical studies on oral microbiome: A practical guide for planning, performing, and reporting. Periodontol 2000. 2021;85:210-236.

Appendix

Additional methods

Collection of samples for microbiome analyses

After isolation of the first molar of the fourth quadrant with cotton rolls and gauzes, and removal of supragingival dental plaque, subgingival dental plaque was collected from this tooth using a sterile universal curette. If the first molar of the fourth quadrant was absent or no sufficient amount of subgingival plaque could be collected, the other molars and premolars of the lower jaw were used in a standardized order. The plaque sample was then centrifuged into 50 µl of RNAprotect (RNAprotect Bacteria Reagent, Qiagen, Venlo, The Netherlands) in a sterile Eppendorf vial.

To collect a stimulated saliva sample, participants were asked to chew paraffin and continuously spit into a sterile collection tube until a total of 5 ml was collected. The saliva was vortexed to ensure a homogenous sample, and pipetted into sterile Eppendorf vials.

A sample of the tongue coating was collected by fixating the tongue with a gauze at the tip, and swiping a microbrush (Microtip micro-applicator regular size; Microbrush International, Grafton, Wisconsin, USA) four times over the midline of the dorsum of the tongue. The tip of the microbrush was cut off and placed in a sterile Eppendorf vial with 50 µl of RNAprotect.

All samples were first stored at -40°C, thereafter transported on dry ice, and stored at -80°C until processing.

Sample processing

DNA isolation

The isolation of DNA was performed in batches of 84 samples per sample type. Isolation blanks (kit chemicals), separate sample blank controls (unused microbrushes in Eppendorf vial, sample procedure followed without participant), and negative PCR controls (PCR mix with DNA free water) were added in each batch.

Samples were thawed. Subgingival plaque was centrifuged at 16000 rpm for 2 minutes and, after removal of the supernatant, resuspended with 150 µl Tris-EDTA buffer pH 8 (self-manufactured, chemicals from Merck, Darmstadt, Germany). Saliva samples were vortexed extensively, and 200 µl of the solution was transferred to an assigned well. Tongue coating microbrushes, together with 150 µl of Tris-EDTA buffer, were transferred using sterile forceps. Each sample

was transferred to an assigned well in a 1.1 ml deep-well plate (Axygen Scientific Inc., CA, USA) containing 250 µl 0.1-mm Zirconia beads (BioSpec Products, Inc. Bartlesville, OK, USA), 200 µl of phenol (Rotiphenol, Carl Roth GMBH&Co. KG, Karlsruhe, Germany), and 200 µl of lysis buffer (MagMini DNA isolation kit, LGC Genomics Ltd, Hoddesdon, UK). The deep-well plate was sealed with a silicone lid and placed in a Mini-BeadBeater-96 (BioSpec Products, Bartlesville, OK, USA) for 2 min at 2,100 oscillations/min.

DNA isolation, quantification, and PCR amplification were performed as previously described. [1] All samples, including isolation blanks, and negative PCR controls were included in the final equimolar pool.

Paired-end sequencing of 12 pmol DNA including 25% PhiX was conducted on the MiSeq platform (Illumina, San Diego, CA, USA) using a MiSeq Reagent kit v3 and 2x251 nt.

Data processing

The 16S rDNA reads were merged, quality-filtered, and checked for possible remaining PhiX reads as previously described, [2] with the exception that a maximum of 25 mismatches was used in the overlap region during read merging. The quality-filtered sequences (maximum expected error of 0.5) were denoised using UNOISE3 (usearch v10.0240, 32-bit [3]). Thereafter, the sequences were mapped into zero-radius operational taxonomic units (zOTUs) using usearch global (max accepts 128, max rejects 1024, max hits 1), where the representative (most abundant) zOTU sequences were assigned a taxonomy as previously described [2] using a trimmed version of HOMD v14.51 [4] as the reference database for the RDP-naive Bayesian classifier with minimum confidence of 80%. [5]

Data analysis

Microbiome data were subsampled at 3,500 reads per sample to normalize for unequal sequencing depth, using the QIIME v1.8.0 single_rarefaction.py script. The zOTU abundances were log2 transformed to normalize the data distribution for principal component analyses (PCA). Permutational multivariate analyses of variance (PERMANOVA, using R v3.6.3 [6] and vegan v2.5-6 [7]) were performed for each of the three niches, i.e., plaque, saliva, and tongue coating, to calculate the significance of the compositional differences among the three groups per niche. When a significant difference was found, post-hoc pairwise PERMANOVAs were performed between the groups, and the compositional difference between the groups was assessed using the linear discriminant analysis (LDA) effect size (LEfSe) analysis (less-strict LEfSe with three groups together, separate per niche) with an LDA value threshold of 3.0, to determine the zOTUs that are differentially abundant between the groups. [8] After removing zOTUs with a relative abundance <0.0001, the online Galaxy framework (http://huttenhower.sph.harvard.edu/galaxy) was used to perform the LEfSe analyses. The zOTUs that were identified by LEfSe were additionally tested for differences in relative abundance among the groups with a Kruskal-Wallis test, using the false discovery rate (FDR) to correct for multiple testing. [9] When a significant result was found, differences between the groups were tested with a post-hoc Mann-Whitney U test. The zOTUs with a median relative abundance of ≥0.01 in at least 1 group were further analysed.

To compare groups by microbial diversity of the microbiome samples, the Shannon diversity index and the number of zOTUs per sample (species richness) were compared with the Kruskal-Wallis test. Furthermore, the Bray-Curtis distances per niche between all samples of all pairs, i.e., ERA – at-risk, ERA – control, and at-risk – control, were calculated and compared (uncorrected for other variables) with Mann-Whitney U tests.

Additional results

Sample diversity

Plaque samples

There was no difference in microbiome α-diversity among the groups by the Shannon diversity index (Kruskal-Wallis, p=0.83), nor by number of zOTUs per sample (Kruskal-Wallis, p=0.76) (appendix Table 2). Comparison of the Bray-Curtis distance between all pairs (ERA – at-risk median 0.605; ERA – control median 0.602; at-risk – control median 0.607) also showed no differences (ERA – at-risk vs ERA – control p=0.97; at-risk – ERA vs at-risk – control p=0.65; control – ERA vs control – at-risk p=0.65) (appendix Figure 1-A).

Saliva samples

The microbiome α-diversity among the groups did not differ by the Shannon diversity index (Kruskal-Wallis, p=0.99), nor by number of zOTUs (Kruskal-Wallis, p=0.37) (appendix Table 2). However, the Bray-Curtis distance between the ERA group and the control group (median 0.470) was significantly higher than between the ERA group and at-risk group (median 0.453), and higher than between the control group and at-risk group (median 0.449) (ERA – at-risk vs ERA – control p<0.001; at-risk – ERA vs at-risk – control p=0.74; control – ERA vs control – at-risk p<0.001) (appendix Figure 1-B).

Tongue coating samples

Similar to the other niches, there was no difference among the groups in α-diversity by the Shannon diversity index (Kruskal-Wallis, p=0.31) nor by number of zOTUs (Kruskal-Wallis, p=0.44) (appendix Table 2). Similar to the saliva samples, the Bray-Curtis distance between the ERA group and the control group (median 0.482) was higher than between the ERA group and at-risk group (median 0.462), and higher than between the control group and at-risk group (median 0.460) (ERA – at-risk vs ERA – control p<0.001; at-risk – ERA vs at-risk – control p=0.18; control – ERA vs control – at-risk p=0.001) (appendix Figure 1-C).

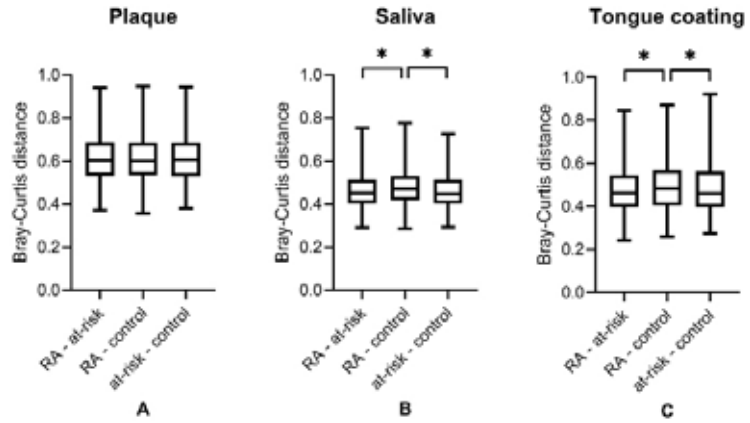
Appendix Table 1. Additional characteristics of the study population.

	ERA group (n=50)	At-risk group (n=50)	Control group (n=50)	*p*
Smoking, yes [n (%)]	12 (24%)	14 (28%)	5 (10%)	0.066[a]
Alcohol, yes [n (%)]	24 (48%)	23 (46%)	24 (48%)	0.974[a]
Drugs, yes [n (%)]	5 (10%)	1 (2%)	3 (6%)	0.242[a]
Painkiller <24 hours [n (%)]	12 (24%)	16 (32%)	9 (18%)	0.265[a]
Antibiotics ≤12 weeks [n (%)]	4 (8%)	8 (16%)	2 (4%)	0.110[a]
Removable (partial) denture [n (%)]	4 (8%)	4 (8%)	3 (6%)	0.907[a]
Tongue cleaning, yes [n (%)]	21 (42%)	23 (46%)	17 (34%)	0.461[a]
Mouth rinse, yes [n (%)]	10 (20%)	6 (12%)	11 (22%)	0.387[a]
Total number of teeth [median (IQR)]	27 (24.8-28)	27 (25-28)	27.5 (25-28)	0.170[b]
DMFT [mean (SD)]	12.82 (7.02)	12.20 (5.92)	11.02 (6.53)	0.375[c]
Time since eating/drinking, hours [median (IQR)]	2.5 (2.0-4.3)	3.5 (2.0-11.3)	3.0 (2.0-4.1)	0.237[b]
Time since oral hygiene, hours [median (IQR)]	18.5 (2.4-25)[1]	24 (21.1-26.3)[2]	24 (10.9-27.6)[3]	0.005[123 b] 0.002[12 d] 0.013[13 d] 0.893[23 d]

n = number of observations, ERA = early rheumatoid arthritis, IQR = Interquartile Range, SD = Standard Deviation, DMFT = decayed, missing, or filled teeth

[a] Chi-square test, [b] Kruskal-Wallis, [c] One-way ANOVA, [d] Mann-Whitney U

[1] ERA group, [2] at-risk group, [3] control group



Appendix Figure 1. Bray-Curtis distances between the early rheumatoid arthritis (ERA) group, at-risk group, and control group in (A) plaque, (B) saliva and (C) tongue coating. Asterisks indicate a significant difference.

Appendix Table 2. Microbiome α-diversity according to Shannon diversity index and number of zero-radius operational taxonomic units (zOTUs) per sample.

	Median (IQR)			
	ERA group	At-risk group	Control group	p-value[1]
Subgingival dental plaque				
Shannon diversity index	3.65 (3.08-3.95)	3.51 (3.00-3.97)	3.60 (3.01-4.00)	0.83
Number of zOTUs per sample	150 (106-184)	139.5 (96.5-174.5)	150 (103-197)	0.76
Stimulated saliva				
Shannon diversity index	3.29 (3.02-3.48)	3.26 (3.07-3.58)	3.30 (2.98-3.55)	0.99
Number of zOTUs per sample	152.5(127.5-179.3)	143 (119-163)	152 (131-173)	0.37
Tongue coating				
Shannon diversity index	2.89 (2.57-3.19)	3.01 (2.69-3.23)	3.14 (2.72-3.31)	0.31
Number of zOTUs per sample	94 (81.5-106)	95 (79-112.5)	102.5 (79.8-119)	0.44

ERA = early rheumatoid arthritis, IQR = Interquartile Range, zOTU = zero-radius operational taxonomic unit

[1] Kruskall-Wallis

Appendix Table 3. Saliva: a summary of the most significant zero-radius operational taxonomic units (zOTUs) that differentiated among the early rheumatoid arthritis (ERA) group, at-risk group, and control group, sorted on linear discriminant analysis (LDA) score per group. Differences in relative abundance among the groups were tested with a Kruskal-Wallis test (false discovery rate corrected level of significance of 0.02), and post-hoc Mann-Whitney U tests. Results for zOTUs with a median relative abundance ≥0.01 for ≥1 group are marked with an outline.

zOTU number	Taxonomy (HOMD)	Group	LDA score	p-value (LEfSe)	p-value (Kruskal-Wallis)	Relative abundance [median (IQR)]			p-value (Mann-Whitney U)
						ERA-group	At-risk group	Control group	
zOTU 2	*Streptococcus salivarius / vestibularis*	ERA	4.21	0.023	0.025				
zOTU 26	*Prevotella veroralis*	ERA	3.55	0.044	0.045				
zOTU 16	*Streptococcus sanguinis*	ERA	3.49	0.022	0.025				
zOTU 25	*Prevotella salivae*	ERA	3.44	0.017	**0.017**	0.010 (0.003-0.020)	0.011 (0.003-0.016)	0.005 (0.001-0.011)	0.667[a], 0.011[b], 0.017[c]
zOTU 17	*Streptococcus*	ERA	3.36	0.034	0.038				
zOTU 35	*Rothia mucilaginosa*	ERA	3.31	0.04	0.042				
zOTU 85	*Veillonella sp._oral_taxon_917*	ERA	3.27	0.0009	**0.001**	0 (0-0.002)	0.001 (0-0.004)	0 (0-0)	0.271[a], 0.008[b], <0.001[c]
zOTU 148	*Selenomonas*	ERA	2.12	0.009	**0.009**	0 (0-0)	0 (0-0)	0 (0-0.0003)	0.006[a], 0.024[b], 0.485[c]
zOTU 56	*Streptococcus downei / sobrinus*	ERA	3.05	0.047	0.048				
zOTU 4	*Veillonella*	At-risk	4.35	0.0003	**<0.001**	0.091 (0.041-0.129)	0.087 (0.054-0.140)	0.051 (0.051-0.083)	0.639[a], 0.002[b], <0.001[c]
zOTU 10	*Prevotella*	At-risk	4.06	0.003	**0.003**	0.024 (0.003-0.051)	0.029 (0.009-0.059)	0.008 (0.001-0.021)	0.269[a], 0.032[b], 0.001[c]
zOTU 14	*Prevotella veroralis*	At-risk	3.66	0.034	0.031				
zOTU 50	*Megasphaera micronuciformis*	At-risk	3.33	0.002	**0.002**	0.005 (0.002-0.010)	0.005 (0.002-0.013)	0.002 (0.000-0.005)	0.445[a], 0.011[b], 0.001[c]
zOTU 857	*Prevotella salivae*	At-risk	3.18	0.005	**0.006**	0 (0-0.0003)	0 (0-0.0003)	0 (0-0)	0.901[a], 0.003[b], 0.005[c]
zOTU 51	*Actinomyces graevenitzii*	At-risk	3.16	0.004	**0.005**	0.002 (0.001-0.005)	0.002 (0.001-0.006)	0.001 (0-0.002)	0.556[a], 0.011[b], 0.002[c]
zOTU 377	*Leptotrichia sp._oral_taxon_212*	At-risk	3.02	0.049	0.049				
zOTU 7	*Neisseria flavescens / subflava*	Control	4.37	0.004	**0.004**	0.023 (0.003-0.052)	0.016 (0.005-0.070)	0.051 (0.019-0.127)	0.798[a], 0.004[b], 0.003[c]
zOTU 1	*Streptococcus dentisani / infantis / mitis / oralis / sp._oral_taxon_058*	Control	4.19	0.029	0.028				
zOTU 15	*Porphyromonas pasteri / sp._oral_taxon_278*	Control	3.96	0.001	**0.001**	0.004 (0.010-0.013)	0.009 (0.001-0.025)	0.022 (0.006-0.057)	0.209[a], <0.001[b], 0.018[c]
zOTU 13	*Fusobacterium periodonticum*	Control	3.73	0.002	**0.002**	0.004 (0.001-0.010)	0.010 (0.001-0.021)	0.015 (0.004-0.033)	0.045[a], <0.001[b], 0.130[c]
zOTU 12	*Veillonella parvula*	Control	3.64	0.0004	**<0.001**	0.011 (0.005-0.020)	0.015 (0.008-0.025)	0.023 (0.015-0.034)	0.258[a], <0.001[b], 0.002[c]
zOTU 38	*Veillonella*	Control	3.34	0.031	0.030				
zOTU 98	*Prevotella nanceiensis*	Control	3.27	0.00003	**<0.001**	0 (0-0.001)	0 (0-0.001)	0.002 (0-0.006)	0.446[a], <0.001[b], <0.001[c]
zOTU 90	*Alloprevotella*	Control	3.16	0.018	0.020				
zOTU 140	*Granulicatella*	Control	3.08	0.002	**0.002**	0 (0-0.001)	0.001 (0-0.004)	0.001 (0-0.003)	0.002[a], 0.002[b], 0.970[c]

zOTU = zero-radius operational taxonomic unit, ERA = early rheumatoid arthritis, HOMD = human oral microbiome database, LDA = linear discriminant analysis, LEfSe = LDA effect size, [a] ERA group versus at-risk group, [b] ERA group versus control group, [c] At-risk group versus control group

Appendix Table 4. Tongue coating: a summary of the most significant zero-radius operational taxonomic units (zOTUs) that differentiated among the early rheumatoid arthritis (ERA) group, at-risk group, and control group, sorted on linear discriminant analysis (LDA) score per group. Differences in relative abundance among the groups were tested with a Kruskal-Wallis test (false discovery rate corrected level of significance of 0.02), and post-hoc Mann-Whitney U tests. Results for zOTUs with a median relative abundance ≥0.01 for ≥1 group are marked with an outline.

zOTU number	Taxonomy (HOMD)	Group	LDA score	p-value (LEfSe)	p-value (Kruskal-Wallis)	Relative abundance [median (IQR)]			p-value (Mann-Whitney U)
						ERA-group	At-risk group	Control group	
zOTU 100	*Lautropia mirabilis*	ERA	3.37	0.028	0.029				
zOTU 16	*Streptococcus sanguinis*	ERA	3.31	0.013	**0.012**	0.001 (0-0.001)	0.002 (0-0.001)	0.0003 (0-0.001)	0.006[a], 0.253[b], 0.036[c]
zOTU 85	*Veillonella sp._oral_taxon_917*	ERA	3.13	0.036	0.036				
zOTU 10	*Prevotella*	At-risk	4.19	0.021	0.022				
zOTU 4	*Veillonella*	At-risk	4.11	0.014	**0.015**	0.084 (0.059-0.111)	0.080 (0.053-0.121)	0.062 (0.015-0.093)	0.924[a], 0.012[b], 0.012[c]
zOTU 51	*Actinomyces graevenitzii*	At-risk	3.64	0.002	**0.002**	0.004 (0.002-0.012)	0.007 (0.002-0.019)	0.002 (0.001-0.005)	0.365[a], 0.009[b], 0.001[c]
zOTU 106	*Mitsuokella sp._oral_taxon_521*	At-risk	3.42	0.035	0.036				
zOTU 50	*Megasphaera micronuciformis*	At-risk	3.40	0.002	**0.001**	0.005 (0.002-0.009)	0.007 (0.003-0.013)	0.003 (0.000-0.005)	0.180[a], 0.017[b], <0.001[c]
zOTU 93	*Leptotrichia sp._oral_taxon_215*	At-risk	3.13	0.041	**0.039**				
zOTU 859	*Veillonella*	At-risk	3.07	0.002	**0.002**	0.0003 (0-0.0003)	0.0003 (0-0.001)	0 (0-0.0003)	0.877[a], 0.002[b], 0.002[c]
zOTU 727	*Streptococcus*	At-risk	3.02	0.014	**0.015**	0 (0-0.0003)	0.0003 (0-0.0003)	0 (0-0.0003)	0.004[a], 0.052[b], 0.324[c]
zOTU 7	*Neisseria flavescens / subflava*	Control	4.35	0.004	**0.004**	0.003 (0.000-0.016)	0.005 (0.002-0.041)	0.035 (0.002-0.100)	0.558[a], 0.002[b], 0.012[c]
zOTU 13	*Fusobacterium periodonticum*	Control	4.29	0.001	**0.001**	0.005 (0.000-0.022)	0.018 (0.001-0.054)	0.042 (0.006-0.090)	0.066[a], <0.001[b], 0.061[c]
zOTU 1	*Streptococcus dentisani / infantis / mitis / oralis / sp._oral_taxon_058*	Control	4.09	0.0001	**<0.001**	0.029 (0.012-0.045)	0.028 (0.014-0.038)	0.045 (0.031-0.077)	0.725[a], 0.001[b], <0.001[c]
zOTU 15	*Porphyromonas pasteri / sp._oral_taxon_278*	Control	3.76	0.013	**0.011**	0.0003 (0-0.005)	0.001 (0-0.005)	0.003 (0-0.019)	0.309[a], 0.003[b], 0.061[c]
zOTU 21	*Granulicatella adiacens*	Control	3.66	0.029	0.032				
zOTU 90	*Alloprevotella*	Control	3.10	0.015	**0.016**	0 (0-0)	0 (0-0.0004)	0 (0-0.002)	0.366[a], 0.006[b], 0.066[c]
zOTU 947	*Streptococcus*	Control	3.09	0.026	0.027				
zOTU 60	*Capnocytophaga sputigena*	Control	3.09	0.011	**0.013**	0 (0-0)	0 (0-0)	0 (0-0.001)	0.847[a], 0.014[b], 0.017[c]

zOTU = zero-radius operational taxonomic unit, ERA = early rheumatoid arthritis, HOMD = human oral microbiome database, LDA = linear discriminant analysis, LEfSe = LDA effect size

[a] ERA group versus at-risk group, [b] ERA group versus control group, [c] At-risk group versus control group

References

1. Kahharova D, Brandt BW, Buijs MJ, Peters M, Jackson R, Eckert G, et al. Maturation of the Oral Microbiome in Caries-Free Toddlers: A Longitudinal Study. J Dent Res. 2020;99:159-167.
2. Koopman JE, Buijs MJ, Brandt BW, Keijser BJ, Crielaard W, Zaura E. Nitrate and the Origin of Saliva Influence Composition and Short Chain Fatty Acid Production of Oral Microcosms. Microb Ecol. 2016;72:479-492.
3. Edgar R. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv. 2016;bioRxiv.081257.
4. Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database (Oxford). 2010;2010:baq013.
5. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73:5261-5267.
6. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. Available from: https://www.R-project.org/.
7. Oksanen J, Guillauma Blanet F, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: Community Ecology Package. R package version 2.5-6. 2019. Available from: https://CRAN.R-project.org/package=vegan.
8. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60.
9. Benjamini YH, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc. 1995;57:289-300.

Oral health-related quality of life

Oral health-related quality of life in patients with early rheumatoid arthritis is associated with periodontal inflammation and painful temporomandibular disorders: a cross-sectional study

Johanna M. Kroese, Catherine M.C. Volgenant, Dirkjan van Schaardenburg, Laurette van Boheemen, Maurits K.A. van Selms, Corine M. Visscher, Wim Crielaard, Bruno G. Loos, Frank Lobbezoo

Published in: Clinical Oral Investigations. 2021. Online ahead of print. PMID: 34279701.

Chapter 07

ABSTRACT

Objectives: To evaluate oral health-related quality of life (OHRQoL) in early rheumatoid arthritis (ERA) patients and individuals at risk of rheumatoid arthritis (RA) compared to healthy controls, and to explore possible associated factors.

Materials and Methods: Fifty ERA patients, 50 at-risk individuals, and 50 age and gender matched healthy controls were recruited. OHRQoL (Oral Health Impact Profile-14; OHIP-14), number of decayed, missing, and filled teeth (DMFT), denture use, periodontal inflamed surface area (PISA), xerostomia (Xerostomia Inventory; XI), and possible TMD(-pain) diagnoses were recorded. The groups were compared on these variables. Subsequently, backward multiple regression analyses were performed for the ERA and at-risk groups, with OHRQoL as the dependent variable, and gender, age, DMFT, denture use, PISA, XI, non-painful TMD, and TMD pain as independent variables.

Results: At-risk individuals had higher XI scores (U=789.5, z=-3.181, p=0.001, r=-0.32) and higher prevalence of TMD pain (p=0.046, OR=4.57; 95% CI 0.92-22.73) than healthy controls, and higher OHIP-14 scores than the ERA group (U=894.5, z=-2.418, p=0.016, r=-0.24), while no difference in OHIP-14 was found between the control group and both other groups. For ERA patients, OHRQoL was associated with PISA and TMD pain (R^2=0.498, p<0.001). For at-risk individuals, OHRQoL was associated with XI score (R^2=0.410, p<0.001).

Conclusions: Alertness of health professionals to TMD pain and periodontal inflammation in ERA patients, and to xerostomia and TMD pain in at-risk individuals is recommended.

Background

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints that causes pain and can result in functional disability and in lower health-related quality of life (HRQoL). [1] Since pharmacological treatment can alleviate symptoms and even prevent joint destruction, there is international consensus on the importance of early identification and treatment. [2] Several characteristics of RA, e.g., arthralgia and the presence of specific autoantibodies, can already be present before the development of clinical arthritis, and thus individuals at risk of RA can be identified. [3]

While HRQoL covers a broad scope, the OHRQoL (*oral* health-related quality of life) reflects the subjective perception of local orofacial conditions on quality of life. [4] Like HRQoL, OHRQoL is a valuable patient-reported outcome measure (PROM) that provides insight into subjective disease burden. Current literature describes an association between RA and several orofacial aspects, e.g., periodontitis, xerostomia, and temporomandibular disorders (TMD) [5-8] – all of which can negatively influence the OHRQoL. [9-14] In addition, other orofacial aspects, e.g., the number of decayed, missing, and filled teeth (DMFT), and use of a denture, may affect OHRQoL. [15] Although previous studies do show a lower OHRQoL in patients with RA compared to healthy controls, only limited data is available on the timeframe around RA onset. [16]

The importance of PROMs in RA is increasingly recognized in research and care, [17-19] making QoL a relevant health outcome. Information on OHRQoL and possible associated orofacial conditions, in early RA (ERA) and in individuals at risk of RA, could highlight orofacial aspects that require targeted treatment. Hence, such information could provide a direction for customized care to limit orofacial inconveniences during an important timeframe. The aim of this study is therefore to evaluate the OHRQoL in patients with ERA and at-risk individuals compared to healthy controls, and to explore possible associated orofacial factors. We hypothesize a higher prevalence of periodontal disease, xerostomia, and TMD in ERA patients, and consequently lower OHRQoL, compared to healthy controls. Like other characteristics of RA, orofacial inconveniences might already occur before RA onset, and thus similar results are expected for the at-risk individuals.

Methods

Study design and ethical approval

This cross-sectional study is part of a larger longitudinal cohort study. A full description of the study protocol has been published previously. [20] The study protocol has been approved by the accredited Medical Ethical Committee of the Slotervaart Hospital and Reade (METc Slotervaartziekenhuis en Reade, U/17.056/P1719), and has been registered in the Dutch National Trial Register (NTR, NTR6362).

Participants and recruitment

Three groups of participants were recruited: (1) patients with ERA, (2) individuals at risk of RA, and (3) a control group with no autoimmune conditions. Groups 1 and 2 were recruited at Reade, a rheumatology clinic in Amsterdam, The Netherlands. Group 1 consisted of patients diagnosed with RA according to the 2010 ACR/EULAR RA criteria [2] within the previous year. For group 2, participants were recruited from the Reade at-risk cohort. [21-23] Participants in this cohort have the combination of inflammatory-type arthralgia and increased serum levels of rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA). For the current study, most participants in group 2 were included on the same day as inclusion in the Reade at-risk cohort, with a few exceptions of up to a maximum of six months hereafter. Participants for group 3 were recruited at the Academic Centre for Dentistry Amsterdam (ACTA), irrespective of oral status, and were matched to groups 1 and 2 regarding sex and age (±5 years). All participants were ≥18 years, had a minimum of 12 natural teeth, and gave written informed consent. Venous blood was collected from all participants to determine serum levels of RF and ACPA, including participants in the control group to rule out a possible unknown increased risk of RA. Individuals with RF levels of >5.0 kU/l and/or ACPA levels of >10.0 kU/l were considered seropositive. All clinical examinations were performed by a single, experienced dentist (JMK).

Outcome variables

Oral health-related quality of life

The shortened Oral Health Impact Profile (OHIP-14) questionnaire was used to measure OHRQoL. [4] The OHIP-14 is derived from the original 49-item OHIP, of which a Dutch translation has been developed and validated, [24] and has been reported adequate for replacing the original 49-item OHIP. [25] With the OHIP-14, the frequency of a variety of possible social impacts of oral disorders (14 questions) during the past month is scored on a five-point Likert scale, ranging from 0 (“never”) to 4 (“very often)”, resulting in a sum score ranging from 0 (no impact) to 56 (maximum impact of the oral health on quality of life).

Oral status

Participants were asked if they currently experienced intra-oral pain. Participants were also asked if they experienced any difficulties when performing oral hygiene during the past week, on a four-point Likert scale ranging from 0 (“without any difficulty”) to 3 (“unable to do”). In case of a score other than 0, a further explanation was asked to identify possible joint problems related to RA. Data were analysed as either having no difficulties (score 0), or having difficulties (score 1 to 3) performing oral hygiene.

An intra-oral inspection was performed to determine the total number of teeth present, and the number of decayed, missing and filled teeth (DMFT), [26] using the International Caries Detection and Assessment System (ICDAS) score [27] of three or more as cut-off value for caries presence, comparable to the WHO caries criteria. [28] The presence or absence of a removable (partial) denture was also recorded. Intra-oral soft tissues were inspected to detect possible mucosal abnormalities, e.g., wounds, abscesses, or fistulas.

Periodontal health

A periodontal examination included registration of bleeding on probing (BOP, present/absent), pocket probing depth (PPD) in millimeters, and positive gingival recession in millimeters on six sites for each tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual). Recording of BOP resulted in a full mouth BOP percentage. The total periodontal inflamed surface area (PISA) was calculated using the method described by Nesse et al., [29] to quantify the total burden of periodontal inflammation.

Xerostomia

To measure subjective symptoms of dry mouth, all participants were asked to fill in the Xerostomia Inventory (XI) questionnaire. [30] The XI is validated in several populations, including a Dutch population, with a forward-back-translation to validate the Dutch translation of the original questionnaire. [31,32] The XI contains 11 questions about the frequency in which someone had to act on, or had trouble functioning because of, the adverse consequences of xerostomia during the past four weeks. All questions are scored on a five-point Likert scale, ranging from 1 (“never”) to 5 (“very often”), resulting in a sum score ranging from 11 (no dry mouth) to 55 (extremely dry mouth).

Temporomandibular disorders

A thorough description of the methods and results concerning classification of possible TMD has been previously described. [33] In brief, the presence of TMD was classified according to the Diagnostic Criteria for TMD (DC/TMD), [34] and five diagnostic categories were recognized: (1) myalgia, (2) arthralgia, (3) disc displacement, (4) degenerative joint disease, and (5) headache attributed to TMD. All participants filled out the DC/TMD symptom questionnaire with 14 questions on pain in the joint area, headache, joint sounds, and joint locking. [35] The clinical examination was performed according to the DC/TMD Clinical Examination Protocol. [36] Results were analysed for having non-painful TMD diagnoses (disc displacement, degenerative joint disease, or both), and for having a TMD-pain diagnoses (myalgia, arthralgia, or both). To be diagnosed with a headache attributed to TMD, a myalgia and/or arthralgia diagnosis is required, and thus this category was not separately analysed.

Statistical analysis

Characteristics of the study population were described using descriptive statistics. First, normal distribution of variables was tested with the Kolmogorov-Smirnov test. For normally distributed variables, the mean (and standard deviation; SD) is reported; otherwise, the median (and interquartile range; IQR) is reported. For normally distributed continuous variables, one-way ANOVA with post-hoc independent samples t-tests was used when comparing the means of the three groups; otherwise, the Kruskal-Wallis test was used, with post-hoc Mann-Whitney U tests. Differences between groups on binary variables were tested with a Chi-square test, or Fisher's exact test when appropriate. Probability levels of less than 0.05 were considered statistically significant.

Subsequently, to evaluate possible associations between OHRQoL and orofacial conditions, a backward multiple linear regression analysis was performed for the ERA group and the at-risk group. The decision to use this type of analysis was based on the advantage of considering the possible effects, and importance of these effects, of all variables simultaneously. The analysis used OHRQoL as the dependent variable, and the following independent variables: gender, age, DMFT, use of a removable (partial) denture, PISA, XI, non-painful TMD, and TMD pain. First, as a preselection procedure, the unadjusted associations with the independent variables were tested. Variables that showed at least a weak association ($p<0.10$) were then included in the multiple regression model. A backward approach was used, where the variables with the weakest predictive value were removed step-by-step, until all independent variables showed at least a p value <0.05 in the final model. From the regression analyses, significant associated factors and their correlation coefficients were extracted.

Variables were tested for multicollinearity based on the variance inflation factor (VIF), and all VIF values were between 1 and 5 and thus considered as inconsequential correlations. [37] All analyses were performed using the IBM SPSS Statistics 26 software package (IBM Corp, Armonk, NY, USA).

Results

Sample demographics and orofacial variables

From November 2017 until July 2019, a total number of 150 participants were included, 50 per group. Unless specifically described otherwise below, data on the measured variables were available for all 150 participants. Table 1 displays the characteristics of the study population. There was no difference in average age or gender distribution between the groups. In the ERA group, patients were included an average of 3.1±1.7 months after being diagnosed with RA.

Oral status and periodontal health

No significant differences were found between the three groups on number of teeth present, DMFT, prevalence of using a removable (partial) denture, or prevalence of currently present intra-oral pain (Table 1). The reported intra-oral pain was of dental origin in 15 cases, of periodontal origin in seven cases, and originating from the intra-oral soft tissues in three cases. Due to the overlap with DMFT and periodontal health, the currently present intra-oral pain variable was not added to the regression analyses.

There also was no difference between the groups in number of participants that reported difficulties with performing oral hygiene during the past week due to physical complaints in joints of the hands and/or arms (Table 1). Interestingly, an additional five participants in the ERA group reported that they used to have difficulties with performing oral hygiene, but this was resolved after starting with the pharmacological treatment for RA.

Further, no differences were found for the investigated periodontal variables, i.e., BOP, average PPD, and PISA (Table 1).

Xerostomia

Forty-nine participants in the at-risk group, and all participants in the ERA-group and control group completed the XI questionnaire. The median total XI score of the at-risk group was significantly higher, indicating more subjective xerostomia, compared to the control group ($U=789.5$, $z=-3.181$, $p=0.001$, $r=-0.32$) (Table 1). No difference was found between the ERA group and the other two groups (Table 1).

Table 1. Demographics of the study sample and results on dental status, periodontal health, xerostomia, and oral health-related quality of life.

	ERA group (n=50)	At-risk group (n=50)	Control group (n=50)	p	Post-hoc, p
Age, years [mean (SD)]	52.1 (13.2)	51.4 (10.3)	51.2 (11.0)	NS[1a]	
Gender, female [n (%)]	39 (78%)	38 (76%)	38 (76%)	NS[1b]	
RF or ACPA positive [n (%)]	38 (76%)	50 (100%)	0 (0%)	N/A	
Dental status					
Total number of teeth [median (IQR)]	27.0 (24.8-28.0)	27.0 (25.0-28.0)	27.5 (25.0-28.0)	NS[1c]	
DMFT [mean (SD)]	12.8 (7.0)	12.2 (5.9)	11.0 (6.5)	NS[1a]	
Removable (partial) denture [n (%)]	4 (8%)	4 (8%)	3 (6%)	NS[1b]	
Intra-oral pain [n (%)]	10 (20%)	11 (22%)	4 (8%)	NS1[b]	
Difficulties with oral hygiene [n (%)]	4 (8%)	8 (16%)	2 (4%)	NS1[b]	
Periodontal health					
BOP (%) [median (IQR)]	19.3 (9.9-35.4)	15.4 (7.4-32.5)	17.5 (8.5-27.5)	NS[1c]	
Average PPD, mm. [median (IQR)]	2.2 (2.0-2.6)	2.2 (2.0-2.5)	2.1 (1.9-2.5)	NS[1c]	
PISA, mm2 [median (IQR)]	258.3 (108.4-398.3)	191.3 (72.5-431.8)	181.4 (91.2-369.4)	NS[1c]	
Xerostomia					
XI score [median (IQR)]	19 (16-25)	22 (17-27)	17 (13-21)	**0.005**[1c]	0.11[2d], 0.81[3d], **0.001**[4d]

ERA = early rheumatoid arthritis, n = number of observations, SD = standard deviation, RF = rheumatoid factor, ACPA = anti-citrullinated protein antibodies, IQR = Interquartile range, DMFT = decayed missing and filled teeth, BOP = bleeding on probing, PPD = pocket probing depth, PISA = periodontal inflamed surface area, XI = xerostomia inventory
Significant results are shown in bold font (p<0.05).
[a] One-way ANOVA, [b] Chi-square test, [c] Kruskal-Wallis, [d] Mann-Whitney U
[1] Three groups, [2] ERA group versus at-risk group, [3] ERA group versus control group, [4] At-risk group versus control group

Temporomandibular disorders

A thorough description on results for TMD (pain) in the study population has been previously reported [33]. A summary of the results is shown in Figure 1. In brief, the three groups did not differ when comparing them on the total number of TMD diagnoses or when comparing them on non-painful TMD diagnoses. However, when considering TMD-pain diagnoses only – either myalgia, arthralgia, or both – participants in the at-risk group more often received a TMD-pain diagnosis than those in the control group (p=0.046, OR=4.57; 95% CI 0.92-22.73). No difference was found between the ERA group and the control group.

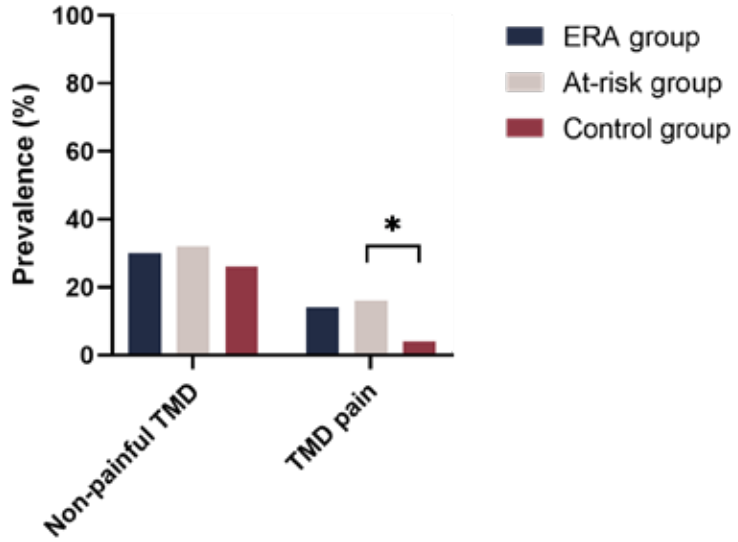


Figure 1. Prevalence of non-painful TMD and TMD pain in patients with early rheumatoid arthritis (ERA), individuals at risk of RA, and healthy controls. An asterisk indicates a significant difference.

Oral Health-Related Quality of Life

Forty-nine participants in the ERA group, and all participants in the at-risk group and the control group completed the OHIP-14 questionnaire. Distributions of the total OHIP-14 scores per group are shown in Figure 2. The median OHIP-14 score of the at-risk group was significantly higher, indicating lower OHRQoL, compared to the ERA group (U=894.5, z=-2.418, p=0.016, r=-0.24). No difference was found between the ERA group or at-risk group compared to the control group (p=0.116 and p=0.248, respectively).

To explore potential factors that were associated with OHRQoL, we performed further analyses. The results of the single and multiple linear regression analyses on OHRQoL for the ERA group and at-risk group are shown in

Tables 2 and 3, respectively. In the ERA group, using a removable (partial) denture, XI score, PISA, and TMD pain showed at least a weak association (p<0.10) with the OHIP-14 score during the preselection. In the backward multiple regression analysis, only PISA (p<0.001) and TMD pain (p<0.001) remained significant. The model predicted 49.8% of the outcome, and both PISA and TMD pain showed a positive correlation to the OHIP-14 score. This means that both an increase in PISA and presence of TMD pain result in a higher OHIP-14 score, and thus lower OHRQoL. The standardized beta suggests a slightly larger effect of TMD pain than of PISA on QoL.

In the at-risk group, sex, age, XI score, and TMD pain showed at least a weak association (p<0.10) with the OHIP-14 score during the preselection. However, according to the backward multiple regression model, only the XI score significantly predicted the OHIP-14 score (p=0.001) in this population. The model predicted 41% of the outcome.

Table 2. Association of orofacial conditions with oral health-related quality of life (OHRQoL) in the group of patients with early rheumatoid arthritis (ERA) (n=50).

	Single	**Backward multiple regression**				
Independent variable	*p value*	*p-to-exit value*	*p value*	*Regression coefficient*	*95% CI*	*Standardized Beta*
Gender (f/m)	0.975					
Age (years)	0.176					
DMFT	0.367					
Use of removable (partial) denture (y/n)	**0.060**	0.123				
PISA	**<0.001**		**<0.001**	0.012	0.006-0.017	0.445
XI	**0.016**	0.140				
Non-painful TMD	0.485					
TMD pain	**<0.001**		**<0.001**	11.456	6.791-16.121	0.518

OHRQoL = oral health-related quality of life, ERA = early rheumatoid arthritis, DMFT = decayed missing and filled teeth, PISA = periodontal inflamed surface area, XI = xerostomia inventory, TMD = temporomandibular disorders, CI = confidence interval
Significant results are shown in bold font (p<0.10 in preselection, p<0.05 in multiple regression).
Multiple regression: R^2 = 0.498, p<0.001.

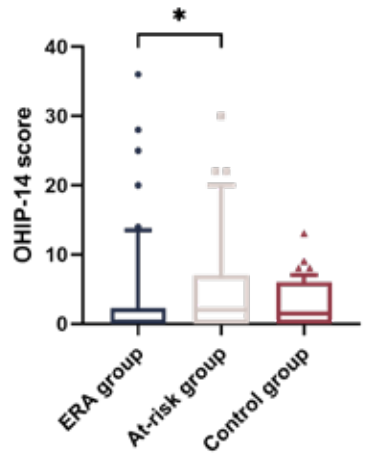


Figure 2. Oral health-related quality of life (OHRQoL) according to the OHIP-14 score in patients with early rheumatoid arthritis (ERA), individuals at risk of RA, and healthy controls. A Kruskall-Wallis test showed a significant result (p=0.042). Post-hoc Mann-Whitney U tests showed a significant result between the ERA group and at-risk group (p=0.016, indicated by an asterisk), but not between the ERA group and at-risk group compared to the control group (p=0.116 and p=0.248, respectively).

Table 3. Association of orofacial conditions with oral health-related quality of life (OHRQoL) in the group of individuals at risk of developing rheumatoid arthritis (RA) (n=49).

	Single	**Backward multiple regression**				
Independent variable	*p value*	*p-to-exit value*	*p value*	*Regression coefficient*	*95% CI*	*Standardized Beta*
Gender (f/m)	0.045	0.665				
Age (years)	0.051	0.203				
DMFT	0.71					
Use of removable (partial) denture (y/n)	**0.772**					
PISA	**0.207**					
XI	**<0.001**		0.001	0.525	0.340-0.710	0.641
Non-painful TMD	0.641					
TMD pain	**<0.001**	0.087				

OHRQoL = oral health-related quality of life, ERA = early rheumatoid arthritis, DMFT = decayed missing and filled teeth, PISA = periodontal inflamed surface area, XI = xerostomia inventory, TMD = temporomandibular disorders, CI = confidence interval
Significant results are shown in bold font (p<0.10 in preselection, p<0.05 in multiple regression).
Multiple regression: R^2 = 0.410, p<0.001.

Discussion

Participants in the at-risk group experienced lower oral health-related quality of life (OHRQoL) compared to the patients in the early rheumatoid arthritis (ERA) group. Within the at-risk group, xerostomia was the only variable that was associated with OHRQoL, with an increase in xerostomia resulting in lower OHRQoL. Furthermore, the median xerostomia index (XI) score was higher, indicating more xerostomia, in the at-risk group compared to the control group. Although challenging to prevent or treat, these results indicate that health professionals should be alert to xerostomia complaints in individuals at risk of RA.

Since the prevalence of TMD pain in the at-risk group was significantly higher than in the control group, alertness to TMD pain in at-risk individuals is also recommended, as previously described. [33] Although the single regression analysis showed a strong association between TMD pain and OHRQoL in the at-risk group, TMD pain was excluded from the multiple regression model. This might be caused by the effect of xerostomia eliminating the effect of TMD pain when considering all variables simultaneously. However, due to the low number of participants further discussed in the limitations below, the possibility that insufficient data was available to identify a significant effect of TMD pain in the multiple regression model should also be considered.

For the ERA group, lower OHRQoL was associated with TMD pain and higher periodontal inflamed surface area (PISA). Both prevalence of TMD pain and that of PISA were not higher compared to the control group. However, the negative effect on OHRQoL suggests that both TMD pain and periodontal inflammation require attention in some patients. Screening for both conditions in patients with ERA could lead to timely treatment and, consequently, alleviation of discomfort.

Interestingly, the results of this study indicate different associated factors for OHRQoL in ERA patients and at-risk individuals. TMD pain was found to be significantly associated with OHRQoL in ERA patients, while in the at-risk group the multiple regression analysis did not identify TMD pain as a significant influence on OHRQoL. In both groups, non-painful TMD was not associated with OHRQoL, indicating that only painful TMD negatively influences QoL.

Further, while PISA was found to be an associated factor in ERA, it did not pass the preselection for the regression analysis in at-risk individuals. Although not significant, numbers on periodontal variables were higher for ERA patients compared to both the at-risk group and the control group, possibly resulting in a more profound effect on OHRQoL.

Contrarily, XI score was found to be significantly associated with OHRQoL in at-risk individuals, but not in ERA patients. The XI is intended as a continuous scale to reduce the risk of misclassification error by using an arbitrary cut-off. [30] However, in a study were both subjective and objective dry mouth were measured, the median XI score in people with normal salivation was reported to be 22.5, while for low salivation and hyposalivation median XI scores of 25 and 39, respectively, were reported. [38] Considering these median scores, XI scores in the current study population are overall relatively low, and prevalence of clinically relevant xerostomia in the ERA group might thus be too low to detect an association with OHRQoL.

Previous studies found poorer dental status and periodontal health in patients with RA compared to healthy controls. [6,39,40] The current study cannot confirm these results, possibly because only RA patients that were very early in the disease were included. Poorer oral health could be caused by impaired oral hygiene due to RA disease activity in the joints of the hands, but the results of this study show little self-reported difficulty in performing oral hygiene. A relation between systemic RA inflammation and inflammation of the periodontal tissues has also been suggested. [41,42] It is imaginable that the effects are not yet present during the first few months since diagnosis, because tooth decay and chronic periodontal inflammation usually require more time to develop. Furthermore, most ERA patients received pharmacological treatment for RA, which can have a positive effect on periodontal inflammation. [42] Our results also indicate a positive effect of pharmacological treatment on oral hygiene, since five ERA patients reported resolving of their difficulties with performing oral hygiene since the start of the pharmacological treatment for RA.

Strengths and limitations

This study reports on OHRQoL and associated factors in a very specific population within the timeframe around RA onset, i.e., patients with ERA and individuals at risk of RA. Consequentially, it required a considerable amount of time to include 50 participants per group. For regression analyses, approximately ten participants are required for each independent variable that is tested. Therefore, a preselection was performed before building the multiple linear regression model. Although a total number of 50 participants per group is thus marginal, it was enough considering the number of variables that were eventually entered into the model. The relatively low number of participants and overall low median OHIP-14 scores, which correspond to OHRQoL in the general Dutch population [43] and a group of type 2 diabetes patients in the Amsterdam area, [44] do imply that

results are based on a limited number of participants with a high OHIP-14 score and thus low OHRQoL.

In RA patients with longer disease duration, OHRQoL was found to be lower than in healthy controls, [16] but the current study cannot confirm this for patients early in the disease. This possibly is because also no differences were found in dental status, periodontal inflammation, TMD pain, and xerostomia – all of which could lower OHRQoL – compared to the healthy control group. It is also imaginable that the burden of a newly diagnosed general disease, and consequently doctor visits and medication, could overshadow orofacial inconveniences.

Further, the XI score was used to measure xerostomia, which only illustrates the subjective perception of dry mouth. For future research, clinical measurement could add valuable information on objective dry mouth, for example by measuring salivary flow rate and intra-oral examination using the Clinical Oral Dryness Scale. [38]

Conclusion

No difference was found when comparing the OHRQoL of ERA patients and at-risk individuals to healthy controls. Although periodontal inflammation and TMD pain were not more prevalent in ERA patients compared to healthy controls, they do negatively influence the OHRQoL, and screening by health professionals for both conditions is thus recommended. In individuals at risk of RA, alertness to xerostomia and TMD pain is recommended, since prevalence of both conditions is higher compared to healthy controls, and xerostomia negatively influences the OHRQoL.

References

1. Matcham F, Scott IC, Rayner L, Hotopf M, Kingsley GH, Norton S, et al. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: a systematic review and meta-analysis. Semin Arthritis Rheum. 2014;44:123-130.
2. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62:2569-2581.
3. van Boheemen L, van Schaardenburg D. Predicting Rheumatoid Arthritis in At-risk Individuals. Clin Ther. 2019;41:1286-1298.
4. Slade GD, Spencer AJ. Development and evaluation of the Oral Health Impact Profile. Community Dent Health. 1994;11:3-11.
5. Aliko A, Alushi A, Tafaj A, Lela F. Oral mucosa involvement in rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis. Int Dent J. 2010;60:353-358.
6. Silvestre-Rangil J, Bagan L, Silvestre FJ, Bagan JV. Oral manifestations of rheumatoid arthritis. A cross-sectional study of 73 patients. Clin Oral Investig. 2016;20:2575-2580.
7. Crincoli V, Anelli MG, Quercia E, Piancino MG, Di Comite M. Temporomandibular Disorders and Oral Features in Early Rheumatoid Arthritis Patients: An Observational Study. Int J Med Sci. 2019;16:253-263.
8. Pantoja LLQ, de Toledo IP, Pupo YM, Porporatti AL, De Luca Canto G, Zwir LF, et al. Prevalence of degenerative joint disease of the temporomandibular joint: a systematic review. Clin Oral Investig. 2019;23:2475-2488.
9. Ferreira MC, Dias-Pereira AC, Branco-de-Almeida LS, Martins CC, Paiva SM. Impact of periodontal disease on quality of life: a systematic review. J Periodontal Res. 2017;52:651-665.
10. Patel RR, Richards PS, Inglehart MR. Periodontal health, quality of life, and smiling patterns--an exploration. J Periodontol. 2008;79:224-231.
11. Naito M, Yuasa H, Nomura Y, Nakayama T, Hamajima N, Hanada N. Oral health status and health-related quality of life: a systematic review. J Oral Sci. 2006;48:1-7.
12. Dahlström L, Carlsson GE. Temporomandibular disorders and oral health-related quality of life. A systematic review. Acta Odontol Scand. 2010;68:80-85.
13. Papagianni CE, van der Meulen MJ, Naeije M, Lobbezoo F. Oral health-related quality of life in patients with tooth wear. J Oral Rehabil. 2013;40:185-190.
14. Karaman A, Sadry S. Evaluation of temporomandibular disorders and oral health-related quality of life with obese patients. Cranio. 2019:1-8.
15. Baniasadi K, Armoon B, Higgs P, Bayat AH, Mohammadi Gharehghani MA, Hemmat M, et al. The Association of Oral Health Status and socio-economic determinants with Oral Health-Related Quality of Life among the elderly: A systematic review and meta-analysis. Int J Dent Hyg. 2021;19:153-165.
16. Schmalz G, Patschan S, Patschan D, Ziebolz D. Oral-Health-Related Quality of Life in Adult Patients with Rheumatic Diseases-A Systematic Review. J Clin Med. 2020;9:1172.
17. Strand V, Wright GC, Bergman MJ, Tambiah J, Taylor PC. Patient Expectations and Perceptions of Goal-setting Strategies for Disease Management in Rheumatoid

Arthritis. J Rheumatol. 2015;42:2046-2054.
18. Kaneko Y, Takeuchi T, Cai Z, Sato M, Awakura K, Gaich C, et al. Determinants of Patient's Global Assessment of Disease Activity and Physician's Global Assessment of Disease Activity in patients with rheumatoid arthritis: A post hoc analysis of overall and Japanese results from phase 3 clinical trials. Mod Rheumatol. 2018;28:960-967.
19. Van der Elst K, Verschueren P, Stouten V, Pazmino S, De Groef A, De Cock D, et al. Patient-Reported Outcome Data From an Early Rheumatoid Arthritis Trial: Opportunities for Broadening the Scope of Treating to Target. Arthritis Care Res (Hoboken). 2019;71:1566-1575.
20. Kroese JM, Volgenant CMC, van Schaardenburg D, Loos BG, Crielaard W, Lobbezoo F. Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol. BDJ Open. 2020;6:7.
21. van Beers-Tas MH, Marotta A, Boers M, Maksymowych WP, van Schaardenburg D. A prospective cohort study of 14-3-3eta in ACPA and/or RF-positive patients with arthralgia. Arthritis Res Ther. 2016;18:76.
22. Holla JFM, van Beers-Tas MH, van de Stadt LA, Landewe R, Twisk JWR, Dekker J, et al. Depressive mood and low social support are not associated with arthritis development in patients with seropositive arthralgia, although they predict increased musculoskeletal symptoms. RMD Open. 2018;4:e000653.
23. van Beers-Tas MH, Blanken AB, Nielen MMJ, Turkstra F, van der Laken CJ, Meursinge Reynders M, et al. The value of joint ultrasonography in predicting arthritis in seropositive patients with arthralgia: a prospective cohort study. Arthritis Res Ther. 2018;20:279.
24. van der Meulen MJ, John MT, Naeije M, Lobbezoo F. The Dutch version of the Oral Health Impact Profile (OHIP-NL): Translation, reliability and construct validity. BMC Oral Health. 2008;8:11.
25. van der Meulen MJ, John MT, Naeije M, Lobbezoo F. Developing abbreviated OHIP versions for use with TMD patients. J Oral Rehabil. 2012;39:18-27.
26. Gruebbel A. A Measurement of Dental Caries Prevalence and Treatment Service for Deciduous Teeth. J Dent Res 1944;23:163-168.
27. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. Community Dent Oral Epidemiol. 2007;35:170-178.
28. Braga MM, Oliveira LB, Bonini GA, Bonecker M, Mendes FM. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization criteria. Caries Res. 2009;43:245-249.
29. Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. J Clin Periodontol. 2008;35:668-673.
30. Thomson WM, Chalmers JM, Spencer AJ, Williams SM. The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health. 1999;16:12-17.
31. Thomson WM, Williams SM. Further testing of the xerostomia inventory. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89:46-50.

General discussion

Chapter 08

This thesis contributes to the knowledge on the interaction between rheumatoid arthritis (RA) and the orofacial system around RA onset. In this general discussion, first, the results of the thesis, and possible areas of interest for interprofessional collaboration are elaborated for the temporomandibular joint (TMJ) and the oral cavity. Subsequently, the longitudinal results of the ongoing observational study are anticipated on. Finally, recommendations for future research are proposed.

The temporomandibular joint

The majority of chapters in this thesis are based on an observational study, designed to investigate several orofacial aspects during the timeframe around RA onset, i.e., in early RA (ERA) patients and individuals at risk of RA. In the at-risk group, all participants were seropositive for rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA). In the ERA group, both seropositive and seronegative patients were included. To fully explore possible TMJ involvement, all temporomandibular disorders (TMD) were considered, i.e., pain and dysfunction of the TMJ and the surrounding masticatory muscles, as well as TMJ sounds during function. The standardized diagnostic criteria for TMD (DC/TMD) [1] were used to determine possible TMD diagnoses.

In *chapter 3*, the prevalence of TMD in ERA patients, at-risk individuals, and healthy controls are presented and compared. When considering all TMD categories together, both painful and non-painful, no differences in the prevalence of TMD among the groups were found. However, the findings in *chapter 3* do show an increased prevalence of painful TMD, that is, myalgia and/or arthralgia, in individuals at risk of RA compared to healthy controls. Unfortunately, since the observational study was the first to investigate TMD in this specific group of individuals, there is no literature to confirm or disprove the current results. Nevertheless, as a conclusion, alertness of all involved health care providers to TMD pain in at-risk individuals is advised.

In ERA patients, the prevalence of TMD pain was not significantly higher than in healthy controls. However, *chapter 7* shows that TMD pain does negatively influence the oral health-related quality of life (OHRQoL) in ERA patients that do experience TMD pain. Further, the results of *chapter 4* show that in RA patients with TMJ involvement, while TMJ pain occurs early in the disease, dysfunction and structural changes develop over time. Although the structural changes were determined based on the presence of an anterior open bite, which is a clinical measure, the results are supported by a study showing the association between established RA and osteoarthritic changes in the TMJ as diagnosed by cone-

beam computed tomography. [2] There is considerable focus on early diagnosis and treatment of RA to prevent irreversible structural damage to any joint in the body, [3] as emphasized by the revised RA classification criteria. [4] Based on the results in this thesis, a similar consideration regarding the TMJ specifically would be appropriate.

Some ERA patients seemed to benefit from the systemic RA treatment on TMD pain, with a decrease or resolving of the pain since the start of the pharmacological treatment, which corresponds to the results of an earlier study in ERA patients. [5] This possible positive effect of systemic treatment on TMD pain mainly seemed to benefit the seropositive patients, which supports the need for specific attention to seronegative patients. However, there are still patients, including seropositive ones, with persisting TMD pain despite the use of systemic treatment. [6,7] Further, the results in *chapter 3* did not show an association between TMD pain and RA disease activity according to the DAS28 (disease activity score based on 28 joints [8]). This indicates that TMD pain can occur regardless of the general disease status, and thus also in patients that in general respond well to the systemic treatment. This corresponds to another recent study on TMJ involvement in RA patients, with significantly more radiographic changes – mostly condylar erosion – in the TMJ compared to the control group, but no significant relation of these radiographic changes to the DAS28 score. [9] The latter authors also suggest that successful treatment with (pain) medication possibly causes oversight of degenerative TMJ problems, [9] which further supports the need for consideration of the TMJ by the involved health care professionals.

In addition to official DC/TMD pain diagnoses, that require familiar pain during the clinical examination and reporting of pain on the DC/TMD symptom questionnaire, *chapter 3* also presents results on unfamiliar pain and non-painful symptoms during the clinical TMJ examination. The high prevalence of these symptoms in both ERA patients and at-risk individuals is relevant, since this could be an early sign of later TMD-pain complaints. [10] Further, painful symptoms in itself may also be a burden to an individual, just as pain complaints negatively influence OHRQoL (see *chapter 7*). A literature review on musculoskeletal symptoms and bruxism pointed to the need for future studies to increase focus on non-painful symptoms, and the need to develop standardized criteria for their assessment. [11]

In *chapter 3*, a possible association between bruxism and TMD was investigated. Due to the relatively low number of participants, the prevalence of awake bruxism was too low for statistical analysis. For sleep bruxism, a significant association with TMD pain in the study population was found, corresponding to findings in other populations. [12-14] However, as highlighted by a recent scoping review on this topic, results are highly dependent on the assessment methods used. It is suggested that sleep bruxism should not be approached with a dichotomous presence/absence outcome, but rather as a multifaceted motor behavior that must be evaluated in its continuum spectrum. [15]

For the above topics, both non-painful symptoms and bruxism, this thesis provides interesting preliminary results in ERA patients and at-risk individuals, but future studies are needed and should adhere to developments in both fields.

Interprofessional collaboration

As described in the general introduction in *chapter 1*, the original DAS was replaced by the DAS28, [8] and with this the TMJ is no longer periodically examined. As discussed above, although no increased prevalence of TMD pain was found in ERA, the results described in this thesis do suggest that attention to the TMJ is necessary for some RA patients. By screening early in the disease, irreversible damage to the TMJ may be prevented and patient burden decreased. Because of the possible TMJ involvement and increased prevalence of TMD in established RA, several studies already emphasize the importance of early detection to be able to provide early management, but these studies often lack specific suggestions for clinical practice. [6,16-18]

In this thesis, the section on pain of the DC/TMD symptom questionnaire is suggested as a useful tool for initial screening on TMD pain in a rheumatology care setting. As discussed in *chapter 3*, the positive predictive value of the DC/TMD symptom questionnaire was 68% for TMD-pain diagnoses in the study population. With a maximum of four questions to be answered – and only one if the answer to the first question is negative – it is a quick, simple, and valid screening tool to identify people that could benefit from further TMD examination and management. Subsequently, people could be referred to a dentist, preferably one with a specialization in orofacial pain and dysfunction.

Anticipating on the further management of these patients, *chapter 5* shows a potential value of corticosteroid injections in the TMJ. Temporarily resulting in lower pain levels and an increased maximum mouth opening capacity, corticosteroid injections could facilitate further treatment with, for example, jaw exercises. It is, however, important to realize that the injections were

administered in a clinical care setting, and other forms of TMD treatment were not taken into account when analyzing the effect of the corticosteroid injections. The effect of these injections in combination with other treatment modalities thus deserves further investigation. In addition, no information was available on systemic RA treatment, and patients were included before therapeutic biologic drugs were introduced as a common treatment modality, which may also affect the TMJ. This means that the results can only be generalized to RA patients that are not on biologic therapy, and the possible effect of biologicals on TMD pain requires further investigation.

It is certainly possible that systemic treatment may reduce TMD pain, as indicated by the results in *chapter 3* and discussed earlier. However, this does not mean that no consideration of the TMJ is needed, since both the results in *chapter 3* on ERA patients, and the existing literature on established RA, still show occurrence of TMD with a prevalence of up to 91%. [6] Since the ERA patients in *chapter 3* were included in the study at an average timespan of three months after the start of the systemic treatment, the results do suggest that a possible effect of systemic treatment on TMD pain can be expected early. As for the timing of screening, it is thus recommended to start soon after the start of the pharmacological RA treatment. As presented in *chapter 4*, the majority of TMJ symptoms seem to start within a few years after RA diagnosis, but the onset of symptoms can also occur at a later stage. Periodical screening should therefore be considered, for example combined with a regular annual consultation within the rheumatology care setting, and by using the suggested DC/TMD symptom questionnaire.

In terms of cost-benefit ratio, a screening for TMD pain with a short questionnaire adds minimal costs and patient burden, and when symptoms are present, referral to a dentist and treatment of affected individuals potentially alleviates pain, prevents structural damage to the TMJ, and increases quality of life. Nevertheless, the positive predictive value of the questionnaire of 68% indicates that approximately one third of patient referrals would be unjustified, resulting in unnecessary costs for either the patient or the national health system, depending on the local insurance policies. Since the DC/TMD symptom questionnaire was used in a population of ERA patients in *chapter 3*, it would be relevant to investigate the possible predictive value of the questionnaire in patients with established RA. Further, to prevent unnecessary referrals and costs, a short clinical examination of the TMJ in patients with a positive questionnaire result could be considered. Such an examination could, for example, be performed by a rheumatology nurse in addition to a routine DAS28 examination, to prevent additional appointments and limit patient burden. Because of the relatively low prevalence of TMD pain found in ERA patients, the results of this thesis do not justify adding a dentist to the multidisciplinary team of a rheumatology care setting. An opportunity for interprofessional collaboration may thus mainly lie in regular communication with, and referral to a qualified oral health care professional. However, with a reported prevalence of TMD of up to 91% in established RA, [6] it would be relevant to further explore the possibility and potential role for a dentist in the multidisciplinary rheumatology care setting.

The oral cavity

This thesis contains findings on several aspects within the oral cavity. Similar to the data on TMD, intra-oral aspects were compared between ERA patients, at-risk individuals, and healthy controls. In *chapter 7*, no differences in the presence of dental plaque and caries were found among the groups, in contrast to earlier studies on RA patients. [19-21] Further, there was no difference in the total number of teeth among the groups, while in established RA a lower number of total teeth was found compared to healthy controls. [9,21,22] Although age might be a confounding factor, a significant relation of tooth loss with established RA was also found in younger adults. [23]

The lack of significant differences in dental status among the three study groups in *chapter 7* may be explained by the fact that tooth decay, and eventually tooth loss, need more time to develop, and are thus not yet found early in the disease. A possible explanation for worse dental status and tooth loss in established RA is an increased difficulty in self-performing oral hygiene. In contrast to patients with established RA, [24] *chapter* 7 reports no difficulties in performing oral hygiene for ERA patients. The results also indicate a positive effect of the systemic RA treatment, since some patients reported resolution of their difficulties with performing oral hygiene after the start of the pharmacological treatment for RA. This is supported by earlier findings that show a relationship between RA disease activity, reported difficulties with performing oral hygiene, [25] and objective status of oral hygiene. [20] However, in the course of RA disease, flare-ups and periods of increased joint complaints may occur, which may cause impaired oral hygiene performance, and consequently worse dental status later on with the disease progression.

Prevalence of xerostomia, the subjective perception of dry mouth, according to the xerostomia inventory (XI) was also reported in *chapter 7*. No difference was found between ERA patients and healthy controls, again in contrast to

earlier findings in patients with established RA. [26,27] However, at-risk individuals reported more xerostomia compared to the control group, and xerostomia was associated with lower OHRQoL. In general, saliva plays an important role in the protection against oral diseases, [28] and xerostomia is an indication of reduced saliva production. Although difficult to treat, attention of the involved health care professionals to possible xerostomia in at-risk individuals is thus recommended.

Periodontitis

The observational study described in this thesis also included measurement of variables related to periodontal inflammation. RA and periodontitis share some characteristics, both being inflammatory diseases that eventually result in destruction of bone tissue. Both diseases also share risk factors, e.g., smoking, obesity, [29-31] and genetic risk factors. [32] It is thus no surprise that earlier studies found an epidemiological relation between RA and periodontitis; as described in the general introduction in *chapter 1*, both an increased risk of periodontitis in RA patients [33] and an increased risk of RA in persons with periodontitis were found. [34] Further, RA disease activity according to the DAS28 and periodontitis severity were found to be associated. [35,36]

However, in *chapters 6* and *7*, although severe periodontitis was found in some ERA patients, the prevalence was not increased compared to healthy controls. Similar to the dental status, it is possible that periodontal disease manifests at a later stage, since it needs more time to develop. This seems a plausible explanation, since patients were classified as having severe periodontitis according to the community periodontal index of treatment needs (CPITN) score 4, requiring a pocket probing depth (PPD) of 6 mm or more, which indeed needs time to develop. Further, the periodontal inflamed surface area (PISA) was evaluated, which also depends on PPD. Again, impaired performance of oral hygiene may play a role in this context. This is supported by a study in ERA patients that found an association between higher RA disease activity and progression of clinical attachment loss over a follow-up period of one year, [37] combined with the earlier mentioned relationship between disease activity, reported difficulties with self-performing oral hygiene, and objective status of oral hygiene.

Another suggestion is that the systemic inflammation and inflammation within the oral cavity are interrelated on a physiological level, and periodontitis may even play a role in RA disease onset. Therefore, actually a higher prevalence of periodontal disease in ERA patients and at-risk individuals compared to healthy controls in the observational study was hypothesized. In *chapter 6*, possible explanations for the unanticipated lack of significant differences for the prevalence of periodontitis among the groups are discussed. In the control group, the prevalence of periodontitis corresponds to that of the worldwide adult population, [38] and the results thus seem representative. However, the prevalence of periodontitis in ERA patients may be underestimated due to selection bias; patients with oral health problems may have declined to participate in the study due to, for example, dental anxiety. Further, the possible influence of systemic medication on periodontal parameters should be considered. Corticosteroid treatment in the ERA patients may have masked active inflammation of the periodontal tissues, and consequently lowered PISA scores, since bleeding on probing (BOP) is part of the PISA equation. Results on the effect of systemic RA treatment on periodontal variables are contradictory, but the use of the (biological) disease-modifying antirheumatic drugs may also result in lower BOP. [36,39-41] The results for at-risk individuals, not showing an increased prevalence of periodontitis compared to healthy controls, also are in contrast with earlier studies. [42,43] Here, as discussed in *chapter 6*, the differences in case definition of periodontitis may play a role.

Altogether, the results in this thesis do not support a possible role for periodontitis in RA development, as often hypothesized. Nonetheless, although not significant, a certain trend was seen towards a higher prevalence of periodontitis in ERA patients and at-risk individuals compared to healthy controls. Additionally, as periodontal inflammation was associated with lower OHRQoL in ERA patients (*chapter 7*), this condition does require attention in some patients. Further, due to the cross-sectional nature of the data, solid conclusions cannot yet be drawn, as further discussed in the section on longitudinal study results.

The oral microbiome

This thesis also considered the oral microbiome in ERA patients and at-risk individuals. As described in *chapter 1*, it is hypothesized that the possible role for oral mucosal sites in RA onset may be attributed to certain characteristics of the oral microbiome. Originally, the hypothesis was that *Porphyromonas gingivalis (P. gingivalis)*, a bacterium associated with periodontal disease, might trigger an RA-associated immune response due to its capability to citrullinate proteins. Although there is a lack of firm, *in vivo* evidence to support this hypothesis, recent narrative reviews continue to discuss this matter, [44] mainly referring to earlier reviews rather than original studies that present actual evidence. The results in *chapter 6* do not support a possible important role for *P. gingivalis*, since overall low abundances and no differences in abundance among the three groups were found.

Further, recent studies stress that it is important to focus on the microbiome as a whole, rather than focusing on specific bacteria. This is supported by the results in *chapter 6*, which show similarities between ERA patients and at-risk individuals, both presenting with a higher relative abundance of possible pro-inflammatory species compared to healthy controls. The increased relative abundance of the genus *Prevotella* corresponds to earlier findings on the oral microbiome in both ERA [45] and established RA, [46] and to studies on the gut microbiome in both ERA [47] and at-risk individuals. [48] *Prevotella* may promote systemic inflammation in response to *Prevotella*-induced mucosal inflammation, [49] or even translocate from the oral cavity to joint tissues to locally induce inflammation. [50] Altogether, these results suggest a possible role for certain inflammophilic genera in the oral microbiome in RA onset. However, as discussed in *chapter 6*, a reversed pathway also needs to be considered; *Prevotella* species may find ecological advantages to emerge at larger numbers due to active systemic inflammation. The currently available literature is insufficient to fully comprehend the possible biological mechanisms and establish a causal link, [50] and due to the cross-sectional nature of the data in this thesis, also no causal conclusions can be drawn. Further, although microbiome dysbiosis may play a role in the development and pathogenesis of RA, it is important to realize that the cause of RA is multifactorial.

Interprofessional collaboration

As described earlier, attention of the involved health care professionals to possible xerostomia is recommended in individuals at risk of RA. Although no increased prevalence of xerostomia was found in ERA patients, earlier studies do show xerostomia in established RA. Referral to a dentist for regular check-ups and oral hygiene instructions may compensate for the increased risk of oral diseases caused by reduced saliva production. Within a rheumatology care setting, screening for xerostomia would be possible with the XI. However, containing eleven questions, it is more time consuming than the DC/TMD symptom questionnaire proposed for TMD-pain screening. A possible more efficient solution could be the summated xerostomia inventory-Dutch version (SXI-D), which contains only five questions and is already validated in several populations. [51]

Based on the results of this thesis, concern about tooth decay or periodontitis is not indicated during the timeframe around RA onset. However, since increased prevalence of oral diseases and difficulties with oral hygiene were found in established RA, [33] it may be relevant to discuss these matters with patients early in the disease. Patients could seek professional oral care when needed, but active referral from the rheumatology care setting may also be justified: based on the current knowledge, the timeframe around RA onset could after all be considered a window of opportunity for the prevention of periodontitis, tooth decay, and tooth loss later when the disease progresses. The results of a recent observational study – although preliminary and in need of further investigation – show that treatment or prevention of periodontitis may even have a positive effect on cardiovascular disease status in RA, due to an association of atherosclerosis and immunoreactivity against periodontitis-associated pathogens, [52] further supporting active referral to an oral health care professional.

Based on the specific findings in the oral microbiome in this thesis, it is too early to conclude on possibilities for interprofessional collaboration, due to the cross-sectional nature of the data. However, if a certain constellation of the oral microbiome indeed plays a role in RA onset, it could thus be a target for RA disease prevention, as described in the anticipation on longitudinal study results below.

Longitudinal study results

Most chapters in this thesis are based on cross-sectional data of the observational study of which the study protocol is presented in *chapter 2*. As described in this protocol, the study also includes a longitudinal aspect, with a follow-up period of three years. The main aim of this longitudinal data collection is to identify possible orofacial predictors for arthritis development in individuals at risk of RA. An earlier study with a cohort of at-risk individuals reported, that the arthritis-free survival curve flattens at approximately 36 months, [53] supporting a follow-up of at least three years. Since the longitudinal study is still ongoing, it is not yet justified to analyze and discuss the preliminary and incomplete results. Nevertheless, possible results and corresponding consequences are anticipated and discussed below.

TMD (pain) is one of the investigated potential orofacial predictors of arthritis development in at-risk individuals. In *chapter 3*, a significantly higher prevalence of TMD pain in at-risk individuals compared to healthy controls, and a high prevalence of non-painful TMJ symptoms in at-risk individuals were found. As arthralgia in the upper and lower extremities adds to the prediction of arthritis development, [53] a predictive value of TMD pain may also be anticipated. Further, as non-painful symptoms were shown to be a possible predictor for later TMD pain, [10] it would be relevant to evaluate whether a similar predictive value of non-painful symptoms is present in specific study populations, in this

case ERA patients and at-risk individuals. The increasing interest in identifying individuals at risk of RA, and possible predictors for arthritis development in these individuals, is mainly based on the potential of disease prevention. While interventions focusing on predictors might eventually lead to the prevention of RA development, only predictors that play a causal role in RA development would be suitable as targets for prevention. Since no causal role for TMD (pain) in arthritis development in at-risk individuals is hypothesized, a possible predictive value of TMD (pain) would not lead to the development of preventive measures. It may, however, add precision to earlier composed prediction models, [53,54] and thus contribute to patient information, and the identification of high-risk patients.

Of greater interest in the context of disease prevention are possible predictors within the oral cavity. Although no increased prevalence of periodontal inflammation was found in at-risk individuals compared to healthy controls, a possible role in RA onset should not be dismissed without evaluating the longitudinal study results. In an earlier study on treatment-naive arthralgia patients with a follow-up of two years, periodontitis at baseline was associated with arthritis activity and future requirement of treatment with methotrexate. [55] With regard to the longitudinal results of the ongoing follow-up, it will therefore be relevant to compare periodontal variables between individuals that do, and individuals that do not develop arthritis within the at-risk group.

Anticipating on possible outcomes, if periodontitis proves to plays a role in the development of RA, it would of course be relevant to investigate the possible preventive value of non-surgical periodontitis treatment, i.e., professional subgingival debridement and optimizing oral self-care, possibly supplemented with antimicrobials such as chlorhexidine or antibiotics. Non-surgical treatment of periodontitis was already reported to have a positive effect on disease activity in other chronic diseases, such as diabetes [56] and cardiovascular diseases. [57] In established RA, a decrease in disease activity and systemic inflammatory markers after non-surgical periodontitis treatment was also reported. [58,59] Recently, it was even suggested that, based on the relationship between periodontitis and RA disease activity, periodontitis should be treated before the evaluation of RA disease activity, in order to avoid unnecessary therapeutic modifications. [60]

The similarities in oral microbiome composition between ERA patients and at-risk individuals, both containing increased relative abundance of possible pro-inflammatory species compared to healthy controls, point towards a role for microbiome dysbiosis in arthritis development. This corresponds to the suggestions and indications in earlier studies. However, cross-sectional data does not provide information on the direction of a possible association. As discussed above and in *chapter 6*, it is not certain whether the microbiome of an individual induces RA onset, or systemic inflammatory activity allows for different species to emerge. The longitudinal results may provide some additional information on this topic, for example if a profound difference will be found in oral microbiome composition between individuals that do, and individuals that do not develop arthritis within the at-risk group. It would also be of interest to analyze both subgroups within the at-risk group in comparison to the ERA patients and healthy controls. Similarities between ERA patients and at-risk individuals that develop arthritis would further support a possible role for the oral microbiome in RA onset. Nevertheless, further research would still be necessary to investigate the precise biological mechanism of this possible role.

In terms of disease prevention, microbiome dysbiosis could for example be targeted with probiotic treatment. Regarding oral diseases, studies suggest a possible role for probiotics in the management of periodontal disease [61] and in the prevention of caries. [62] Further, for gut microbiome dysbiosis in RA, studies already show that probiotics might be an effective approach. While up to now clinical studies mainly focused on the possible effect of probiotics on RA-related pathophysiological parameters in established RA, preclinical studies show promising preventive results for probiotics with *Lactobacillus* species, with reductions in pro-inflammatory cytokines and an increase of anti-inflammatory cytokines. [63] However, the oral and gut microbiomes may require a different approach due to differences in stability and resilience, as demonstrated by a radically different response to antibiotics. [64]

Recommendations for future research

As described in the previous section, longitudinal data of the ongoing study may provide more information on the possible role of orofacial aspects in the onset of RA. However, some limitations of the study should be considered. The main limitation of this study is the relatively low number of participants, with 50 participants in each group. Since both ERA patients and at-risk individuals are a very specific population, it took a considerable amount of time to include these 50 participants per group. Further, not all newly diagnosed RA patients at the center for rheumatology agreed to participate, possibly due to the (psychosocial) burden of being diagnosed, already resulting in multiple visits and the start of pharmacological treatment. However, the results of the study do indicate areas of interest for further research, and can give direction to larger studies.

Instead of performing an extensive clinical orofacial and intra-oral examination in a limited amount of participants, it could be considered to start with screening on a larger scale, for example with the DC/TMD symptom questionnaire and the XI. Further, quite some research is emerging using self-reported questionnaires for periodontitis, which facilitates the screening of larger study populations. [65-67] This may increase participation rate, due to the small time investment required and low burden for participants. Subsequently, a subset of people could be recruited for further, more time-consuming clinical examination, e.g., the clinical part of the DC/TMD, and an intra-oral examination including periodontal examination and the clinical oral dryness score (CODS) to examine objective dry mouth. As discussed in *chapter 3*, bruxism seems to be a relevant factor to consider when evaluating TMD pain in the study population, and should thus also be considered in future research. Currently, there is an ongoing shift in the approach towards bruxism, with developments in definition and diagnosis, [68] and a standardized tool for the assessment of bruxism (STAB) is being developed by an expert panel of professionals from different medical fields. [69] Although still ongoing, it is recommended to follow this evolution of the bruxism construct in both research and patient care.

The possible role of periodontitis and the oral microbiome in the onset of RA is a topic of great interest. However, the reviews on this topic almost seem to outnumber the actual, valuable clinical studies that can contribute to the evaluation and interpretation of this possible relationship. In this thesis, severe periodontitis was classified according to the CPITN score. Research on periodontitis in general displays a wide variety in the case definition of periodontitis, which complicates the comparison of results. In recent years, a new case definition system including a process of staging and grading of periodontitis was proposed. [70] Evaluation of the consistency and accuracy of this system shows satisfactory results. [71] To increase standardization and aid comparison of results, it is thus recommended to use this new case definition system in future research.

For research on the oral microbiome, the results in *chapter 6* point toward the further exploration of saliva and tongue coating microbiome. In contrast with subgingival dental plaque, for which no differences were found among the groups, both saliva and tongue coating are relatively easy to collect. This facilitates research on a larger scale, and microbiome samples would not have to be collected by an oral health care professional. To examine possible shifts in microbiome composition, it would be preferable to collect microbiome samples over time. With this however, researches should realize the increased complexity in terms of logistics, due to long-term preservation of samples at very low temperature, and the necessity to analyze all samples simultaneously.

Finally, another limitation of the current ongoing longitudinal study to consider is that RF-positive, ACPA-negative individuals were also included in the group of individuals at risk of RA. Although RF is associated with RA, it is increasingly recognized that the presence of ACPA displays a far more important predictive value in at-risk individuals. [72] In *chapter 6*, a sub analysis on the oral microbiome for ACPA-positive versus ACPA-negative at-risk individuals did not show any differences. However, including ACPA-negative individuals will result in a smaller number of participants that develop arthritis during follow-up, and consequently less power for the analysis on possible predictors of arthritis development. It is thus recommended to only include ACPA-positive at-risk individuals in future research.

Conclusions

The timeframe around RA onset may be considered a window of opportunity for the prevention of future orofacial complications. Timely recognition and treatment of TMD pain may prevent dysfunction and irreversible damage to the TMJ, while consideration of oral hygiene and oral health may prevent periodontitis, tooth decay, and tooth loss. The results of this thesis do need reinforcement by future studies, and are insufficient to justify adding an oral health professional to every multidisciplinary team in rheumatology care settings. However, screening for TMD and xerostomia in rheumatology care settings is strongly advised, and referral of newly diagnosed ERA patients to a dentist – or at least encouragement of regular dental check-ups – seems justified. Analyses of longitudinal results and future studies are needed to identify possible orofacial targets for RA disease prevention, and subsequent possible opportunities for interprofessional collaborations.

References

1. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. J Oral Facial Pain Headache. 2014;28:6-27.
2. Campos DES, de Araujo Ferreira Muniz I, de Souza Villarim NL, Ribeiro ILA, Batista AUD, Bonan PRF, et al. Is there an association between rheumatoid arthritis and bone changes in the temporomandibular joint diagnosed by cone-beam computed tomography? A systematic review and meta-analysis. Clin Oral Investig. 2021;25:2449-2459.
3. van Dongen H, van Aken J, Lard LR, Visser K, Ronday HK, Hulsmans HM, et al. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum. 2007;56:1424-1432.
4. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-1588.
5. Chin Jen Sem JP, van der Leeden M, Visscher CM, Britsemmer K, Turk SA, Dekker J, et al. Prevalence, Course, and Associated Factors of Pain in the Temporomandibular Joint in Early Rheumatoid Arthritis: Results of a Longitudinal Cohort Study. J Oral Facial Pain Headache. 2017;31:233-239.
6. Kurtoglu C, Kurkcu M, Sertdemir Y, Ozbek S, Gurbuz CC. Temporomandibular disorders in patients with rheumatoid arthritis: A clinical study. Niger J Clin Pract. 2016;19:715-720.
7. Bracco P, Debernardi C, Piancino MG, Cirigliano MF, Salvetti G, Bazzichi L, et al. Evaluation of the stomatognathic system in patients with rheumatoid arthritis according to the research diagnostic criteria for temporomandibular disorders. Cranio. 2010;28:181-186.
8. van Riel PL, Renskers L. The Disease Activity Score (DAS) and the Disease Activity Score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol. 2016;34:S40-S44.
9. Yaman D, Goller Bulut D, Ustaoglu G, Avci E, Tasci M. Dental and temporomandibular joint alterations in rheumatoid arthritis patients and their association with salivary oxidative stress. Turk J Med Sci. 2021. Online ahead of print.
10. Ohrbach R, Bair E, Fillingim RB, Gonzalez Y, Gordon SM, Lim PF, et al. Clinical orofacial characteristics associated with risk of first-onset TMD: the OPPERA prospective cohort study. J Pain. 2013;14:T33-50.
11. Baad-Hansen L, Thymi M, Lobbezoo F, Svensson P. To what extent is bruxism associated with musculoskeletal signs and symptoms? A systematic review. J Oral Rehabil. 2019;46:845-861.
12. Jimenez-Silva A, Pena-Duran C, Tobar-Reyes J, Frugone-Zambra R. Sleep and awake bruxism in adults and its relationship with temporomandibular disorders: A systematic review from 2003 to 2014. Acta Odontol Scand. 2017;75:36-58.
13. Huhtela OS, Napankangas R, Joensuu T, Raustia A, Kunttu K, Sipila K. Self-Reported Bruxism and Symptoms of Temporomandibular Disorders in Finnish University Students. J Oral Facial Pain Headache. 2016;30:311-317.
14. Magalhaes BG, Freitas JLM, Barbosa A, Gueiros M, Gomes SGF, Rosenblatt A, et al. Temporomandibular disorder: otologic implications and its relationship to sleep bruxism. Braz J Otorhinolaryngol. 2018;84:614-619.
15. Manfredini D, Lobbezoo F. Sleep Bruxism and Temporomandibular Disorders: a scoping review of the literature. J Dent. 2021:103711.
16. Byun SH, Min C, Choi HG, Hong SJ. Increased Risk of Temporomandibular Joint Disorder in Patients with Rheumatoid Arthritis: A Longitudinal Follow-Up Study. J Clin Med. 2020;9:3005.
17. Lin YC, Hsu ML, Yang JS, Liang TH, Chou SL, Lin HY. Temporomandibular joint disorders in patients with rheumatoid arthritis. J Chin Med Assoc. 2007;70:527-534.
18. Mortazavi N, Babaei M, Babaee N, Kazemi HH, Mortazavi R, Mostafazadeh A. Evaluation of the Prevalence of Temporomandibular Joint Involvement in Rheumatoid Arthritis Using Research Diagnostic Criteria for Temporomandibular Disorders. J Dent (Tehran). 2018;15:332-338.
19. Manzano BR, da Silva Santos PS, Bariquelo MH, Merlini NRG, Honorio HM, Rubira CMF. A case-control study of oral diseases and quality of life in individuals with rheumatoid arthritis and systemic lupus erythematosus. Clin Oral Investig. 2021;25:2081-2092.
20. Almasi S, Karbalaei Sabbagh M, Barzi D, Tahooni A, Atyabi H, Basir Shabestari S. Relationship between clinical and laboratory findings of rheumatoid arthritis patients with their oral status and disease activity. Caspian J Intern Med. 2021;12:22-28.
21. González-Chávez SA, Pacheco-Tena C, Campos Torres RM, Quiñonez-Flores CM, Reyes-Cordero G, Caraveo Frescas TJ. Temporomandibular and Odontological Abnormalities in Patients with Rheumatoid Arthritis. Reumatol Clin. 2020;16:262-271..
22. de Pablo P, Dietrich T, McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. J Rheumatol. 2008;35:70-76.
23. Kim JW, Park JB, Yim HW, Lee J, Kwok SK, Ju JH, et al. Rheumatoid arthritis is associated with early tooth loss: results from Korea National Health and Nutrition Examination Survey V to VI. Korean J Intern Med. 2019;34:1381-1391.
24. Protudjer JLP, Billedeau C, Hurst K, Schroth R, Stavropoulou C, Kelekis-Cholakis A, et al. Oral Health in Rheumatoid Arthritis: Listening to Patients. JDR Clin Trans Res. 2021:23800844211012678.
25. Radwan-Oczko M, Dus-Ilnicka I, Richards P, Thomsen AM, Rasmussen C. Evaluation of Oral Health Status and Oral Care of Patients with Rheumatoid Arthritis. Int J Dent. 2020;2020:8896766.
26. Crincoli V, Anelli MG, Quercia E, Piancino MG, Di Comite M. Temporomandibular Disorders and Oral Features in Early Rheumatoid Arthritis Patients: An Observational Study. Int J Med Sci. 2019;16:253-263.
27. Silvestre-Rangil J, Bagan L, Silvestre FJ, Bagan JV. Oral manifestations of rheumatoid arthritis. A cross-sectional study of 73 patients. Clin Oral Investig. 2016;20:2575-2580.
28. Llena-Puy C. The role of saliva in maintaining oral health and as an aid to diagnosis.

Med Oral Patol Oral Cir Bucal. 2006;11:E449-455.
29. de Hair MJ, Landewe RB, van de Sande MG, van Schaardenburg D, van Baarsen LG, Gerlag DM, et al. Smoking and overweight determine the likelihood of developing rheumatoid arthritis. Ann Rheum Dis. 2013;72:1654-1658.
30. Arboleda S, Vargas M, Losada S, Pinto A. Review of obesity and periodontitis: an epidemiological view. Br Dent J. 2019;227:235-239.
31. Kumar S. Evidence-Based Update on Diagnosis and Management of Gingivitis and Periodontitis. Dent Clin North Am. 2019;63:69-81.
32. de Molon RS, Rossa C, Jr., Thurlings RM, Cirelli JA, Koenders MI. Linkage of Periodontitis and Rheumatoid Arthritis: Current Evidence and Potential Biological Interactions. Int J Mol Sci. 2019;20:4541
33. Fuggle NR, Smith TO, Kaul A, Sofat N. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis. Front Immunol. 2016;7:80.
34. de Oliveira Ferreira R, de Brito Silva R, Magno MB, Carvalho Almeida A, Fagundes NCF, Maia LC, et al. Does periodontitis represent a risk factor for rheumatoid arthritis? A systematic review and meta-analysis. Ther Adv Musculoskelet Dis. 2019;11:1759720X19858514.
35. Hussain SB, Botelho J, Machado V, Zehra SA, Mendes JJ, Ciurtin C, et al. Is there a bidirectional association between rheumatoid arthritis and periodontitis? A systematic review and meta-analysis. Semin Arthritis Rheum. 2020;50:414-422.
36. Rovas A, Puriene A, Punceviciene E, Butrimiene I, Stuopelyte K, Jarmalaite S. Associations of periodontal status in periodontitis and rheumatoid arthritis patients. J Periodontal Implant Sci. 2021;51:124-134.
37. Heredia PA, Lafaurie GI, Bautista-Molano W, Trujillo TG, Chalem-Choueka P, Bello-Gualtero JM, et al. Predictive factors related to the progression of periodontal disease in patients with early rheumatoid arthritis: a cohort study. BMC Oral Health. 2019;19:240.
38. Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res. 2014;93:1045-1053.
39. Zamri F, de Vries TJ. Use of TNF Inhibitors in Rheumatoid Arthritis and Implications for the Periodontal Status: For the Benefit of Both? Front Immunol. 2020;11:591365.
40. de Smit MJ, Westra J, Posthumus MD, Springer G, van Winkelhoff AJ, Vissink A, et al. Effect of Anti-Rheumatic Treatment on the Periodontal Condition of Rheumatoid Arthritis Patients. Int J Environ Res Public Health. 2021;18:2529.
41. Rinaudo-Gaujous M, Blasco-Baque V, Miossec P, Gaudin P, Farge P, Roblin X, et al. Infliximab Induced a Dissociated Response of Severe Periodontal Biomarkers in Rheumatoid Arthritis Patients. J Clin Med. 2019;8:751.
42. Bello-Gualtero JM, Lafaurie GI, Hoyos LX, Castillo DM, De-Avila J, Munevar JC, et al. Periodontal Disease in Individuals With a Genetic Risk of Developing Arthritis and Early Rheumatoid Arthritis: A Cross-Sectional Study. J Periodontol. 2016;87:346-356.
43. Mankia K, Cheng Z, Do T, Hunt L, Meade J, Kang J, et al. Prevalence of Periodontal Disease and Periodontopathic Bacteria in Anti-Cyclic Citrullinated Protein Antibody-Positive At-Risk Adults Without Arthritis. JAMA Netw Open. 2019;2:e195394.
44. Alghamdi MA, Redwan EM. Interplay of Microbiota and Citrullination in the Immunopathogenesis of Rheumatoid Arthritis. Probiotics Antimicrob Proteins. 2021. Online ahead of print.
45. Scher JU, Ubeda C, Equinda M, Khanin R, Buischi Y, Viale A, et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. Arthritis Rheum. 2012;64:3083-3094.
46. Correa JD, Fernandes GR, Calderaro DC, Mendonca SMS, Silva JM, Albiero ML, et al. Oral microbial dysbiosis linked to worsened periodontal condition in rheumatoid arthritis patients. Sci Rep. 2019;9:8379.
47. Maeda Y, Takeda K. Host-microbiota interactions in rheumatoid arthritis. Exp Mol Med. 2019;51:1-6.
48. Alpizar-Rodriguez D, Lesker TR, Gronow A, Gilbert B, Raemy E, Lamacchia C, et al. Prevotella copri in individuals at risk for rheumatoid arthritis. Ann Rheum Dis. 2019;78:590-593.
49. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology. 2017;151:363-374.
50. Iljazovic A, Amend L, Galvez EJC, de Oliveira R, Strowig T. Modulation of inflammatory responses by gastrointestinal Prevotella spp. - From associations to functional studies. Int J Med Microbiol. 2021;311:151472.
51. Thomson WM, van der Putten GJ, de Baat C, Ikebe K, Matsuda K, Enoki K, et al. Shortening the xerostomia inventory. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112:322-327.
52. Giles JT, Reinholdt J, Andrade F, Konig MF. Associations of Antibodies Targeting Periodontal Pathogens With Subclinical Coronary, Carotid, and Peripheral Arterial Atherosclerosis in Rheumatoid Arthritis. Arthritis Rheumatol. 2021;73:568-575.
53. van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis. 2013;72:1920-1926.
54. Rakieh C, Nam JL, Hunt L, Hensor EM, Das S, Bissell LA, et al. Predicting the development of clinical arthritis in anti-CCP positive individuals with non-specific musculoskeletal symptoms: a prospective observational cohort study. Ann Rheum Dis. 2015;74:1659-1666.
55. Hashimoto M, Yamazaki T, Hamaguchi M, Morimoto T, Yamori M, Asai K, et al. Periodontitis and Porphyromonas gingivalis in preclinical stage of arthritis patients. PLoS One. 2015;10:e0122121.
56. Baeza M, Morales A, Cisterna C, Cavalla F, Jara G, Isamitt Y, et al. Effect of periodontal treatment in patients with periodontitis and diabetes: systematic review and meta-analysis. J Appl Oral Sci. 2020;28:e20190248.
57. Sanz M, Marco Del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and cardiovascular diseases: Consensus report. J Clin Periodontol. 2020;47:268-288.
58. Silva DS, Costa F, Baptista IP, Santiago T, Lund H, Tarp S, et al. Evidence-based research on effectiveness of periodontal treatment in rheumatoid arthritis patients: a systematic review and meta-analysis. Arthritis Care Res (Hoboken). 2021. Online ahead of print.
59. Nguyen VB, Nguyen TT, Huynh NC, Nguyen KD, Le TA, Hoang HT. Effects of non-surgical periodontal treatment in rheumatoid arthritis patients: A randomized

clinical trial. Dent Med Probl. 2021;58:97-105.
60. Manzo C, Kechida M. Periodontitis and rheumatoid arthritis: three messages from published literature to clinical practice. Reumatologia. 2020;58:339-340.
61. Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: Systematic review and meta-analysis. J Dent. 2016;48:16-25.
62. Zaura E, Twetman S. Critical Appraisal of Oral Pre- and Probiotics for Caries Prevention and Care. Caries Res. 2019;53:514-526.
63. Paul AK, Paul A, Jahan R, Jannat K, Bondhon TA, Hasan A, et al. Probiotics and Amelioration of Rheumatoid Arthritis: Significant Roles of Lactobacillus casei and Lactobacillus acidophilus. Microorganisms. 2021;9:1070.
64. Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MP, Rashid MU, et al. Same Exposure but Two Radically Different Responses to Antibiotics: Resilience of the Salivary Microbiome versus Long-Term Microbial Shifts in Feces. mBio. 2015;6:e01693-01615.
65. Verhulst MJ, Teeuw WJ, Gerdes VE, Loos BG. Self-reported oral health and quality of life in patients with type 2 diabetes mellitus in primary care: a multi-center cross-sectional study. Diabetes Metab Syndr Obes. 2019;12:883-899.
66. Verhulst MJL, Teeuw WJ, Bizzarro S, Muris J, Su N, Nicu EA, et al. A rapid, non-invasive tool for periodontitis screening in a medical care setting. BMC Oral Health. 2019;19:87.
67. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Beck JD, et al. Self-reported measures for surveillance of periodontitis. J Dent Res. 2013;92:1041-1047.
68. Manfredini D, Ahlberg J, Lobbezoo F. Bruxism definition: Past, present, and future - What should a prosthodontist know? J Prosthet Dent. 2021;S0022-3913(21)00074-3.
69. Manfredini D, Ahlberg J, Aarab G, Bracci A, Durham J, Ettlin D, et al. Towards a Standardized Tool for the Assessment of Bruxism (STAB)-Overview and general remarks of a multidimensional bruxism evaluation system. J Oral Rehabil. 2020;47:549-556.
70. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89 Suppl 1:S173-S182.
71. Marini L, Tonetti MS, Nibali L, Rojas MA, Aimetti M, Cairo F, et al. The staging and grading system in defining periodontitis cases: consistency and accuracy amongst periodontal experts, general dentists and undergraduate students. J Clin Periodontol. 2021;48:205-215.
72. Bos WH, Wolbink GJ, Boers M, Tijhuis GJ, de Vries N, van der Horst-Bruinsma IE, et al. Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated protein antibody status: a prospective cohort study. Ann Rheum Dis. 2010;69:490-494.

Summary

Samenvatting

Chapter 09

Summary

Rheumatoid arthritis (RA) is an auto-immune disease affecting the synovial joints. Since no cure is available for RA, pharmacological treatment focuses on alleviating symptoms and arresting disease progression. Early intervention results in better clinical outcomes and may even prevent joint destruction. Therefore, there is an important focus on early diagnosis in both research and clinical practice. Since several characteristics of RA can be present before the clinical outbreak of arthritis, e.g., arthralgia and presence of the antibody rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA) in serum, individuals at risk of RA can be identified.

The current literature reports several interactions between RA and the orofacial system, such as an increased prevalence of temporomandibular disorders (TMD) in RA patients. Further, difficulties in performing oral self-care, reduced saliva production, and increased prevalence of dental plaque and caries are reported. For periodontitis, a chronic inflammatory disease of the tooth-supporting tissues, bidirectional associations are found. Furthermore, it has been suggested that the oral microbiome may play a role in RA onset.

While in general there is an important focus on the timeframe around RA onset, current knowledge on possible orofacial complications in the preclinical and early stages of RA disease is very limited. The main aim of this thesis is therefore to increase knowledge on the orofacial system during the timeframe around RA onset.

Chapter 2 is an extensive description of the study protocol of a prospective observational cohort study that was designed to investigate several orofacial aspects in patients with early RA (ERA) and individuals at risk of RA compared to a healthy control group. The protocol aimed at including a total number of 150 participants, i.e., 50 per group. Outcome measures at baseline include TMD, bruxism, the number of decayed, missing, and filled teeth, periodontal status, subjective xerostomia, oral health-related quality of life (OHRQoL), and the collection of oral microbiome samples. The protocol also includes a three-year follow-up to evaluate possible orofacial predictors for RA development in at-risk individuals. Analysis of the longitudinal results stretches beyond the span of this thesis.

In *chapter 3*, the findings of the observational study on the prevalence of TMD and bruxism are reported. Conforming to the protocol, participants were recruited in three groups (50 per group): patients with ERA (included on average 3.1 months after RA diagnosis), at-risk individuals, and healthy controls. A possible TMD diagnosis was determined according to the diagnostic criteria for TMD (DC/TMD). While no differences were found between the groups when considering all TMD diagnoses, at-risk individuals more often had a TMD-pain diagnosis (myalgia and/or arthralgia) than healthy controls (16% versus 4%). No such difference was found when comparing the ERA group to the control group (14% versus 4%). However, within the ERA group, seronegative patients had a TMD-pain diagnosis more often than seropositive patients (33% versus 8%). Further, participants with TMD pain were more often diagnosed with probable sleep bruxism. The results suggest that health professionals should be alert to TMD pain in at-risk individuals and seronegative RA patients, and that sleep bruxism might be an important factor in the development of TMD pain in this population. The DC/TMD symptom questionnaire is suggested as a useful tool to screen for possible TMD in a rheumatology care setting.

Chapter 4 compares ERA patients and patients with established RA on clinical temporomandibular joint (TMJ) symptoms and inflammatory mediators in TMJ synovial fluid in a cross-sectional study design. The clinical examination of eighty joints in 68 RA patients included TMJ pain at rest, maximum mouth opening, palpation, jaw movement capacity, number of painful movements, crepitus, and degree of anterior open bite. TMJ synovial fluid was collected to determine the presence of several inflammatory mediators. In the study population, TMJ symptoms predominantly developed within five years following general symptom onset. ERA (general symptom duration ≤2 years) was associated with higher general pain, higher TMJ pain on maximum mouth opening, greater maximum mouth opening, and crepitus, while established RA was associated with a larger number of involved joints, a larger number of painful movements, anterior open bite, and higher plasma and synovial fluid levels of tumor necrosis factor. The results indicate that TMJ pain and crepitus usually occur early in the disease, while pain-related dysfunction and structural changes develop over time, supporting the importance of early recognition and treatment of TMJ involvement in RA.

Chapter 5 explores the clinical value of corticosteroid injections for the treatment of TMJ pain and dysfunction in patients with RA, in relation to systemic inflammatory activity, in a prospective cohort study design. In 35 RA patients, the clinical examination included maximum mouth opening capacity, degree of anterior open bite, TMJ-pain intensity at rest, and crepitus. After the baseline examination, 53 joints received a corticosteroid (methylprednisolone) injection. The examination was repeated for all patients after a median of 3.1 weeks, and again for 21 patients after a median of 6.3 weeks, of whom 20 patients received a second corticosteroid injection at the second visit. TMJ corticosteroid injections resulted in an increase of maximum mouth opening capacity and a decrease of TMJ-pain intensity, that lasted for approximately three weeks. No correlations were found with systemic inflammatory activity. Corticosteroid injections thus seem useful for the short-term management of TMJ involvement in RA, and could facilitate additional noninvasive, conservative TMD treatment.

In *chapter 6*, the results of the observational study (*chapter 2*) on periodontal health and the oral microbiome are presented. To determine periodontal health, bleeding on probing, pocket probing depth, and periodontal inflamed surface area (PISA) were measured. The microbial composition of subgingival dental plaque, saliva, and tongue coating was assessed using 16S rDNA amplicon sequencing. No differences were found for any of the periodontal variables between ERA patients, at-risk individuals, and healthy controls. Microbiome analyses showed significant differences in microbiome composition among the groups for saliva and tongue coating, but not for plaque. While ERA patients and at-risk individuals showed similarities in microbiome composition, both groups presented with increased relative abundance of potentially pro-inflammatory species compared to healthy controls. The results suggest a possible association between the oral microbiome and RA onset. However, longitudinal results are needed to further explore this possible association.

Chapter 7 reports on the results of the observational study (*chapter 2*) on OHRQoL. First, the results on dental status, periodontal health, subjective xerostomia, and TMD are described. Subsequently, possible associations of these variables with OHRQoL are reported. No differences were found between ERA patients, at-risk individuals, and healthy controls on the total number of decayed, missing, and filled teeth, denture use, and periodontal variables. At-risk individuals had higher xerostomia inventory (XI) scores, indicating more xerostomia, and had a TMD-pain diagnosis more often than healthy controls. Further, OHRQoL in at-risk individuals was lower compared to the ERA patients, while no difference in OHRQoL was found between the control group and both other groups. For ERA patients, OHRQoL was associated with periodontal inflammation and TMD pain. For at-risk individuals, OHRQoL was associated with XI score only. Based

on the results of this study, alertness of health professionals to TMD pain and periodontal inflammation in ERA patients, and to xerostomia and TMD pain in at-risk individuals, is highly recommended.

Finally, *chapter 8* discusses the main findings of the studies included in this thesis. Possible areas of interest for interprofessional collaboration are elaborated, and recommendations for future research are proposed. In conclusion, the timeframe around RA onset may be considered a window of opportunity for the prevention of future orofacial complications. Timely recognition and treatment of TMD pain may prevent dysfunction and irreversible damage to the TMJ, while consideration of oral hygiene and oral health may prevent periodontitis, tooth decay, and tooth loss. The results of this thesis do need reinforcement by future studies, and are insufficient to justify adding an oral health professional to every multidisciplinary team in rheumatology care settings. However, screening for TMD and xerostomia in rheumatology care settings is strongly advised, and referral of newly diagnosed ERA patients to a dentist – or at least encouragement of regular dental check-ups – seems justified. Analyses of longitudinal results and future studies are needed to identify possible orofacial targets for RA disease prevention, and subsequent possible opportunities for interprofessional collaborations.

Samenvatting

Reumatoïde artritis (RA) is een auto-immuunziekte die de synoviale gewrichten aantast. Omdat er geen genezing mogelijk is, is de farmacologische behandeling van RA gericht op het verlichten van symptomen en het remmen van ziekteprogressie. Vroegtijdige interventie resulteert in betere klinische resultaten en kan zelfs schade aan gewrichten voorkomen. Er is daarom veel aandacht voor vroegdiagnostiek in zowel onderzoek als klinische zorg. Omdat verschillende kenmerken van RA, zoals artralgie en verhoogde serumwaardes van reumafactor (RF) en/of antilichamen tegen gecitrullineerde eiwitten (‘anti-citrullinated protein antibody’; ACPA), aanwezig kunnen zijn voorafgaand aan de klinische presentatie van artritis, kunnen personen met een verhoogd risico op RA worden geïdentificeerd.

De recente literatuur beschrijft verschillende interacties tussen RA en het orofaciale stelsel, waaronder een verhoogde prevalentie van temporomandibulaire disfunctie (TMD) bij RA-patiënten. Verder worden problemen bij het zelf uitvoeren van de mondverzorging, verminderde speekselproductie en een verhoogde prevalentie van tandplaque en cariës beschreven. Voor parodontitis, een chronische ontsteking van de weefsels rondom de tanden en kiezen, zijn bidirectionele associaties met RA gevonden. Bovendien wordt gesuggereerd dat het orale microbioom een rol kan spelen bij het ontstaan van RA.

Hoewel er in het algemeen veel aandacht is voor het tijdsbestek rondom het ontstaan van RA, is de huidige kennis over mogelijke orofaciale complicaties in de preklinische en vroege stadia van RA zeer beperkt. Het belangrijkste doel van dit proefschrift is daarom om de kennis over het orofaciale stelsel gedurende het tijdsbestek rondom het ontstaan van RA te vergroten.

In *hoofdstuk 2* wordt een uitgebreide beschrijving gegeven van het onderzoeksprotocol van een prospectieve observationele cohortstudie die is opgezet om verschillende orofaciale aspecten te onderzoeken bij patiënten met vroege RA en personen met een verhoogd risico op RA in vergelijking met een gezonde controlegroep. Het protocol heeft de intentie om een totaal van 150 deelnemers te includeren, namelijk 50 per onderzoeksgroep. Uitkomstmaten op baseline zijn onder meer TMD, bruxisme, het aantal carieuze, ontbrekende en gerestaureerde gebitselementen, de parodontale status, subjectief gevoel van

droge mond (xerostomie), mondgezondheid-gerelateerde levenskwaliteit ('oral health-related quality of life'; OHRQoL) en het verzamelen van orale microbioom-samples. Het protocol beschrijft ook een follow-up van drie jaar om mogelijke orofaciale voorspellers voor de ontwikkeling van RA te evalueren bij personen met een verhoogd risico op RA. Analyse van de longitudinale resultaten valt buiten de strekking van dit proefschrift.

In *hoofdstuk 3* worden de resultaten van de observationele studie betreffende de prevalentie van TMD en bruxisme gerapporteerd. Conform het protocol werden de deelnemers in drie groepen geïncludeerd (50 per groep): patiënten met vroege RA (gemiddeld 3.1 maanden na de diagnose van RA), personen met een verhoogd risico op RA en gezonde controles. Een mogelijke TMD-diagnose werd bepaald aan de hand van de diagnostische criteria voor TMD (DC/TMD). Alhoewel er geen verschil werd gevonden tussen de groepen ten aanzien van alle TMD-diagnoses samen, werd wel vaker een TMD-pijndiagnose (myalgie en/of artralgie) gevonden in personen met een verhoogd risico op RA vergeleken met gezonde controles (16% versus 4%). Een dergelijk verschil werd niet gevonden bij het vergelijken van de vroege RA-groep met de controlegroep (14% versus 4%). Wel hadden binnen de vroege RA-groep seronegatieve patiënten vaker een TMD-pijndiagnose dan seropositieve patiënten (33% versus 8%). Verder werden deelnemers met TMD-pijn vaker gediagnosticeerd met 'waarschijnlijk' slaapbruxisme (bepaald door een combinatie van zelf-rapportage en klinisch onderzoek). De resultaten suggereren dat professionals in de gezondheidszorg alert moeten zijn op TMD-pijn bij mensen met een verhoogd risico op RA en bij seronegatieve RA-patiënten en dat slaapbruxisme een belangrijke factor kan zijn in de ontwikkeling van TMD-pijn bij deze populaties. De DC/TMD-symptomenvragenlijst wordt voorgesteld als een bruikbaar hulpmiddel om te screenen op mogelijke TMD binnen de reumazorg.

In *hoofdstuk 4* worden patiënten met vroege RA en patiënten met gevorderde RA in een cross-sectionele studie vergeleken op klinische symptomen van het kaakgewricht en ontstekingsmediatoren in de synoviale vloeistof van het kaakgewricht. Het klinische onderzoek van 80 kaakgewrichten bij 68 RA-patiënten bestond uit het vaststellen van pijn in rust, bij maximale mondopening en bij palpatie, de kaakbewegingscapaciteit, het aantal pijnlijke bewegingen, crepitatie en de mate van anterieure open beet. Synoviale vloeistof uit het kaakgewricht werd verzameld om de aanwezigheid van verschillende ontstekingsmediatoren te bepalen. In de onderzoekspopulatie ontwikkelde kaakgerichtspijn zich voornamelijk binnen vijf jaar na de start van de algemene RA-symptomen. Vroege RA (symptoomduur ≤2 jaar) was geassocieerd met meer algemene pijn, meer kaakgewrichtspijn bij maximale mondopening, een grotere maximale mondopening en crepitatie, terwijl gevorderde RA geassocieerd was met een groter aantal aangedane gewrichten, een groter aantal pijnlijke kaakbewegingen, een anterieure open beet en een hogere concentratie van tumornecrosefactor in synoviale vloeistof van het kaakgewricht en in plasma. De resultaten laten zien dat kaakgewrichtspijn en crepitatie doorgaans vroeg in de ziekte voorkomen, terwijl pijn-gerelateerde disfunctie en structurele veranderingen in het kaakgewricht zich in de loop van de tijd ontwikkelen, wat het belang van vroege herkenning en behandeling van betrokkenheid van het kaakgewricht bij RA onderstreept.

In *hoofdstuk 5* wordt in een prospectief cohortonderzoek de klinische bruikbaarheid onderzocht van injecties met corticosteroïden voor de behandeling van pijn en disfunctie van het kaakgewricht bij RA-patiënten, in relatie tot systemische ontstekingsactiviteit. Bij 35 RA-patiënten bestond het klinische onderzoek uit het vaststellen van de maximale mondopening, de anterieure open beet, de intensiteit van kaakgewrichtspijn in rust en crepitatie. Na het onderzoek op baseline werden 53 kaakgewrichten geïnjecteerd met corticosteroïden (methylprednisolon). Het klinische onderzoek werd herhaald voor alle patiënten na 3.1 (mediaan) weken en opnieuw voor 21 patiënten na 6.3 (mediaan) weken, van wie 20 patiënten een tweede injectie met corticosteroïden ontvingen bij het tweede bezoek. De injecties in het kaakgewricht resulteerden in een toename van de maximale mondopening en een afname van de pijnintensiteit in het kaakgewricht gedurende ongeveer drie weken. Er werden geen correlaties gevonden met systemische ontstekingsactiviteit. Corticosteroïde-injecties lijken dus waardevol voor de kortetermijnbehandeling van betrokkenheid van het kaakgewricht in RA en zouden aanvullende, niet-invasieve TMD-behandelingen, zoals oefentherapie, kunnen faciliteren.

In *hoofdstuk 6* worden de resultaten van de observationele studie (*hoofdstuk 2*) betreffende de parodontale gezondheid en het orale microbioom gepresenteerd. Om de parodontale gezondheid te bepalen, werden bloeding bij sonderen, pocketdiepte en het totale ontstoken parodontale oppervlak ('periodontal inflamed surface area'; PISA) gemeten. De samenstelling van het orale microbioom van de subgingivale tandplaque, het speeksel en de tongcoating werd vastgesteld met behulp van 16S rDNA-ampliconsequencing. Er werden geen verschillen gevonden voor de parodontale variabelen tussen patiënten met vroege RA, personen met een verhoogd risico op RA en gezonde

controles. Analyses van het orale microbioom toonden significante verschillen aan tussen de groepen voor speeksel en tongcoating, maar niet voor tandplaque. Terwijl de patiënten met vroege RA en personen met een verhoogd risico op RA overeenkomsten lieten zien in de samenstelling van het microbioom, werd in beide groepen een verhoogde relatieve aanwezigheid van potentieel pro-inflammatoire soorten gevonden vergeleken met de gezonde controlegroep. De resultaten suggereren een mogelijk verband tussen het orale microbioom en de ontwikkeling van RA. Er zijn echter longitudinale resultaten nodig om deze mogelijke associatie verder te onderzoeken.

Hoofdstuk 7 beschrijft de resultaten van de observationele studie (*hoofdstuk 2*) betreffende OHRQoL. Eerst worden de resultaten beschreven over de status van het gebit, de parodontale gezondheid, de xerostomie en TMD. Vervolgens worden mogelijke associaties van deze variabelen met OHRQoL beschreven. Er werden geen verschillen gevonden tussen patiënten met vroege RA, personen met een verhoogd risico op RA en gezonde controles bij het vergelijken van het aantal carieuze, ontbrekende en gerestaureerde gebitselementen, de aanwezigheid van een gebitsprothese en de parodontale variabelen. Personen met een verhoogd risico op RA hadden wel een hogere score op de xerostomie vragenlijst ('xerostomia inventory'; XI), wat meer xerostomie impliceert, en vaker een TMD-pijndiagnose dan gezonde controles. Verder was de OHRQoL bij personen met een verhoogd risico op RA lager in vergelijking met vroege RA-patiënten, terwijl geen verschil in OHRQoL werd gevonden tussen de controlegroep en beide andere groepen. Voor vroege RA-patiënten was OHRQoL geassocieerd met parodontale ontsteking en TMD-pijn. Voor personen met een verhoogd risico op RA was OHRQoL alleen geassocieerd met de XI-score. Op basis van de resultaten van dit onderzoek wordt professionals in de gezondheidszorg aanbevolen alert te zijn op TMD-pijn en parodontale ontsteking bij vroege RA-patiënten en op xerostomie en TMD-pijn bij personen met een verhoogd risico op RA.

Tot slot worden in *hoofdstuk 8* de belangrijkste bevindingen besproken van de studies die onderdeel uitmaken van dit proefschrift. Mogelijkheden voor interprofessionele samenwerkingen worden uitgewerkt en aanbevelingen voor toekomstig onderzoek worden gedaan. Concluderend kan het tijdsbestek rondom het ontstaan van RA worden beschouwd als een *window of opportunity* voor de preventie van toekomstige orofaciale complicaties. Tijdige herkenning en behandeling van TMD-pijn kan disfunctie en onomkeerbare schade aan het kaakgewricht voorkomen, terwijl aandacht voor mondhygiëne en mondgezondheid mogelijk parodontitis, cariës en het verlies van gebitselementen kan voorkomen. De resultaten van dit proefschrift behoeven bekrachtiging door toekomstig onderzoek en zijn onvoldoende om de toevoeging van een mondzorgprofessional aan elk multidisciplinair team binnen de reumazorg te rechtvaardigen. Wel wordt screening op TMD en xerostomie binnen de reumazorg sterk aangeraden en lijkt verwijzing van nieuw gediagnosticeerde RA-patiënten naar een tandarts – of ten minste het aanmoedigen van regelmatige tandheelkundige controles – gerechtvaardigd. Analyse van longitudinale resultaten en toekomstige studies zijn nodig om mogelijke orofaciale aangrijpingspunten voor preventie van RA, en daaropvolgende mogelijkheden voor interprofessionele samenwerking, te identificeren.

List of publications
Author contributions
About the author
Dankwoord

Chapter 10

List of publications

Peer reviewed full-text publications

Included in this thesis

Kroese JM, Kopp S, Lobbezoo F, Alstergren P. TMJ Pain and Crepitus Occur Early Whereas Dysfunction Develops Over Time in Rheumatoid Arthritis. J Oral Facial Pain Headache. 2020;34(4):398-405.

Kroese JM, Volgenant CMC, van Schaardenburg D, Loos BG, Crielaard W, Lobbezoo F. Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol. BDJ Open. 2020;6:7.

Kroese JM, Volgenant CMC, Crielaard W, Loos B, van Schaardenburg D, Visscher CM, Lobbezoo F. Temporomandibular disorders in patients with early rheumatoid arthritis and at-risk individuals in the Dutch population: a cross-sectional study. RMD Open. 2021;7(1):e001485.

Kroese JM, Volgenant CMC, van Schaardenburg D, van Boheemen L, van Selms MKA, Visscher CM, Crielaard W, Loos BG, Lobbezoo F. Oral health-related quality of life in patients with early rheumatoid arthritis is associated with periodontal inflammation and painful temporomandibular disorders: a cross-sectional study. Clin Oral Investig. 2021. Online ahead of print.

Kroese JM, Brandt BW, Buijs MJ, Crielaard W, Lobbezoo F, Loos BG, van Boheemen L, van Schaardenburg D, Zaura E, Volgenant CMC. Differences in the Oral Microbiome in Patients With Early Rheumatoid Arthritis and Individuals at Risk of Rheumatoid Arthritis Compared to Healthy Individuals. Arthritis Rheumatol. 2021;73(11):1986-1993.

Kroese JM, Kopp S, Lobbezoo F, Alstergren P. Corticosteroid injections in the temporomandibular joint temporarily alleviate pain and improve function in rheumatoid arthritis. Clin Rheumatol. 2021;40(12):4853-4860.

Other

van Boheemen L, van Beers-Tas MH, **Kroese JM**, van de Stadt LA, van Schaardenburg D, Nurmohamed MT. Cardiovascular risk in persons at risk of developing rheumatoid arthritis. PLoS One. 2020;15(8):e0237072.

Author contributions

Chapter 2 | Temporomandibular joint function, periodontal health, and oral microbiome in early rheumatoid arthritis and at-risk individuals: a prospective cohort study protocol
J.M. Kroese, C.M.C. Volgenant, D. van Schaardenburg, B.G. Loos, W. Crielaard, F. Lobbezoo

Conceived and designed the study:	JMK, CMCV, DvS, BGL, WC, FL
Collected the data:	N/A
Analysed the data:	N/A
Interpreted the data:	N/A
Drafted the manuscript:	JMK
Critically revised the manuscript:	CMCV, DvS, BGL, WC, FL

Chapter 3 | Temporomandibular disorders in patients with early rheumatoid arthritis and at-risk individuals in the Dutch population: a cross-sectional study
J.M. Kroese, C.M.C. Volgenant, W. Crielaard, B. Loos, D. van Schaardenburg, C.M. Visscher, F. Lobbezoo

Conceived and designed the study:	JMK, CMCV, WC, BL, DvS, FL
Collected the data:	JMK
Analysed the data:	JMK
Interpreted the data:	JMK, CMCV, FL
Drafted the manuscript:	JMK
Critically revised the manuscript:	CMCV, WC, BL, DvS, CMV, FL

Chapter 4 | TMJ pain and crepitus occur early whereas dysfunction develops over time in rheumatoid arthritis
J.M. Kroese, S. Kopp, F. Lobbezoo, P. Alstergren

Conceived and designed the study:	JMK, FL, PA
Collected the data:	SK, PA
Analysed the data:	JMK, PA
Interpreted the data:	JMK, FL, PA
Drafted the manuscript:	JMK, PA
Critically revised the manuscript:	SK, FL, PA

Chapter 5 | Corticosteroid injections in the temporomandibular joint temporarily alleviate pain and improve function in rheumatoid arthritis
J.M. Kroese, S. Kopp, F. Lobbezoo, P. Alstergren

Conceived and designed the study:	JMK, FL, PA
Collected the data:	SK, PA
Analysed the data:	JMK, PA
Interpreted the data:	JMK, FL, PA
Drafted the manuscript:	JMK, PA
Critically revised the manuscript:	SK, FL, PA

Chapter 6 | Differences in the oral microbiome in patients with early rheumatoid arthritis and individuals at risk of rheumatoid arthritis compared to healthy individuals
J.M. Kroese, B.W. Brandt, M.J. Buijs, W. Crielaard, F. Lobbezoo, B.G. Loos, L. van Boheemen, D. van Schaardenburg, E. Zaura, C.M.C. Volgenant

Conceived and designed the study:	JMK, WC, FL, BGL, DvS, CMCV
Collected the data:	JMK
Analysed the data:	JMK, BWB
Interpreted the data:	JMK, BWB, BGL, EZ
Drafted the manuscript:	JMK, BWB, MJB
Critically revised the manuscript:	WC, FL, BGL, LvB, DvS, EZ, CMCV

Chapter 7 | Oral health-related quality of life in patients with early rheumatoid arthritis is associated with periodontal inflammation and painful temporomandibular disorders: a cross-sectional study
J.M. Kroese, C.M.C. Volgenant, D. van Schaardenburg, L. van Boheemen, M.K.A. van Selms, C.M. Visscher, W. Crielaard, B.G. Loos, F. Lobbezoo

Conceived and designed the study:	JMK, CMCV, DvS, WC, BGL, FL
Collected the data:	JMK
Analysed the data:	JMK
Interpreted the data:	JMK, CMCV, MKAvS, CMV, FL
Drafted the manuscript:	JMK
Critically revised the manuscript:	CMCV, DvS, LvB, MKAvS, CMV, WC, BGL, FL

About the author

Anna Kroese was born as Johanna Margaretha Kroese in 1987 in Amsterdam, The Netherlands. She graduated as a dentist from the Academic Center for Dentistry Amsterdam (ACTA) in 2011, after which she followed a shortened military training – where she met her better half Rick – and worked as a major-dentist at the Royal Dutch Army for several years. From 2016, she worked as a general dentist, and in 2017 she returned to ACTA to combine her work in a general dental practice with a part time PhD project.

During the past years, she also switched to special care dentistry and completed her specialization in gerodontology, ran a full marathon, wrote and illustrated a children's book (*Letters leren met Sammie en Teske – Een dierenfeestje!*, ISBN 978 90 830730 0 2), and extended her (un)healthy love for cats to her newborn son Kees. Future plans include never running a marathon again and blooming in her new position as assistant professor at the Department of Oral Medicine at ACTA.

Dankwoord

Allereerst veel dank aan de deelnemers aan het klinisch onderzoek; jullie deelname is van onschatbare waarde geweest voor dit proefschrift en daarmee voor mij.

Promotiecommissie
Prof. dr. F. Lobbezoo, beste Frank, door het prettige gesprek met jou vijf jaar geleden ben ik aan dit avontuur begonnen en daar ben ik je nog altijd dankbaar voor. Je bent vriendelijk en toegankelijk, betrouwbaar en serieus, stuurt bij waar nodig en laat vrij. Ik had me geen betere promotor kunnen wensen.

Prof. dr. D. van Schaardenburg, beste Dirkjan, zonder jou was de voor dit proefschrift onmisbare samenwerking met Reade er niet geweest. Jouw blik vanuit de reumatologie was altijd verhelderend en waardevol. Het was daarnaast heel leerzaam om een aantal keer mee te mogen kijken met jouw spreekuur.

Prof. dr. B.G. Loos, beste Bruno, jouw scherpe inzicht en met potlood in de kantlijn gekrabbelde opmerkingen zijn ontzettend waardevol geweest voor de publicaties in dit proefschrift. Inhoudelijk, maar ook taalkundig – ik ben onder de indruk van de manier waarop je in het Engels door met drie woorden te schuiven een zin tien keer beter maakt.

Dr. C.M.C. Volgenant, beste Catherine, van het promotieteam stond jij het dichtste bij. Het is bewonderenswaardig hoe jij jouw verschillende taken op ACTA combineert. Het is dankzij jouw begeleiding dat ik de afgelopen jaren heb geleerd wat het betekent om zelfstandig onderzoek te doen.

Alle overige leden van de promotiecommissie dank ik hartelijk voor de bereidheid mijn proefschrift te beoordelen.

ACTA
De afgelopen jaren maakte ik deel uit van de secties orofaciale pijn en disfunctie, preventieve tandheelkunde en parodontologie op ACTA; teveel collega's om iedereen bij naam te noemen. Ik ben iedereen dankbaar die heeft bijgedragen aan de leerzame en vooral ook ontzettend leuke tijd die mijn promotie is geworden.

Specifieke dank gaat uit naar de co-auteurs op de publicaties in dit proefschrift. Prof. dr. W. Crielaard, beste Wim, vanaf het begin betrokken bij het project, een goed voorbeeld van hoe weinig woorden een waardevolle bijdrage kunnen zijn. Prof. dr. C.M. Visscher, beste Corine, je was vooral betrokken bij de start van het project, nog voordat ik op ACTA begon, maar ook de afgelopen jaren was je altijd op de hoogte, geïnteresseerd en behulpzaam en was jouw input enorm waardevol. Prof. dr. E. Zaura, beste Egija, heerlijk no-nonsens, ik ben dankbaar voor de razendsnelle en ontzettend waardevolle feedback op het artikel over de microbiologie. Maurits, veel dank voor jouw analytische en gedetailleerde blik op de statistiek van het QoL artikel. Bernd, jouw geduld is bewonderenswaardig; letterlijk urenlang beantwoorde je mijn herhaaldelijke vragen over de voor jou zo vanzelfsprekende microbiologie-analyses, waarvoor heel veel dank. Mark, jouw oprechte, belangeloze bereidheid om een ander te helpen – bijvoorbeeld bij geklungel met droogijs – vind ik enorm waardevol.

En dan de dames van 3N.75; Magdalini, Julia, Merel, Loreine, Liza en Bach Van, wat ben ik blij dat ik jullie heb leren kennen! Een geweldige groep onderzoekers, die allemaal aan uiteenlopende onderwerpen werken, maar het enthousiasme voor onderzoek delen. Dankzij jullie was 3N.75 een kamer vol gezelligheid en inspiratie, met daarnaast voldoende koffiepauzes en etentjes voor nog meer gezelligheid en ventilatie.

Reade
Voor de uitvoering van het onderzoek heb ik ook veel tijd doorgebracht op Reade. Laurette, je had uiteraard een belangrijke rol in het onderzoek vanwege je inzet voor het at-risk cohort, maar ook de keren dat we samen hebben gelunch vond ik waardevol en gezellig. Ed en Rick, jullie toverden een ongezellig leeg hoekje om tot mijn eigen onderzoeksruimte, waarvoor veel dank. Alle verpleegkundigen ben ik dankbaar voor de oprechte interesse in mijn onderzoek en de onmisbare en onvermoeibare hulp bij het werven van patiënten. Iedereen bij het bloedafname lab, bedankt voor de hulp als ik materiaal nodig had of met een vraag zat en bedank voor het nauwkeurig verwerken en versturen van de bloedsamples naar het OLVG. En alle dames van de afdeling radiologie waar mijn onderzoeksruimte was ingericht, jullie maakten de vele uren op Reade gezellig. Jullie leefden mee bij no-shows, vierden mee bij mijlpalen en nodigden me uit voor leuke teamuitjes. Ilse, jij in het bijzonder; met mijn onderzoek zat ik jou het meest in de weg. Je had alle reden om dat vervelend te vinden, maar koos voor gezelligheid en ik ben blij dat we nog steeds contact houden.

Malmö
Although relatively short, my time at Malmö University feels like a significant part of my time as a PhD student. Literally everyone at the department contributed

to the wonderful experience I had with every ‘fika’, lunch and quiz night, for which I cannot thank you all enough.

But most of all, prof. P. Alstergren, dear Per, I am grateful to you for giving me the opportunity to be part of your Malmö family. From the moment I arrived, you made me feel at home and welcome. You are serious and professional, but at the same time approachable, humorous, and kind. You have the knowledge and busy schedule that fit your position as a professor, but always found time to sit down and work on our project together. I feel greatly inspired by you.

OLVG
Sabine Wymeersch, Inez-Anne Haagen, Margret de Koning en iedereen van de bloedprikpoli in het OLVG Oost, bedankt dat jullie het mogelijk hebben gemaakt om bloedsamples af te nemen bij de onderzoeksdeelnemers. Dankzij jullie voelde ik me zelfverzekerd genoeg om de venapuncties zelf uit te voeren en werden alle samples snel verwerkt.

Het mooiste vak: tandheelkunde
Iedereen bij de Stip, bedankt voor de leuke tijd die ik bij jullie heb gehad. Najim, eeuwig vrolijk, extreem gepassioneerd voor je vak, ondernemer en leidinggevende, maar ook onderdeel van je team. Het is inspirerend hoe jij je droom waarmaakt en mijn dank is groot voor jouw steun bij mijn onderzoek en de keus om de geriatrie-opleiding in te gaan.

De geriatrie-opleiding die ik volgde bij SBT. Claar, dank dat je zo’n fijne opleider was en wat leuk dat we straks op ACTA weer collega’s zijn. Marion en Cynthia, met jullie aan de andere kant van de stoel was het elke dag een feestje. Pien, je bent top. Margot, jij ook. Teveel collega’s om allemaal te noemen, maar jullie weten dat ik zowel de inhoudelijke samenwerking als de gezelligheid op de werkvloer enorm heb gewaardeerd.

Kiki, Nastaran, Alicia, Nour, Elnaz en Jessica, ik ben ontzettend blij dat het ondanks verschillende woonplaatsen en drukke levens toch lukt elkaar af en toe nog te zien. Jullie zijn echte clinici die laten zien dat je ons mooie vak ook na tien jaar nog met zoveel liefde kan beoefenen, met inmiddels al vijf eigen praktijken in de groep. Het is verfrissend en inspirerend om me af en toe tussen zulke ondernemende powervrouwen te bevinden. En Azadeh, wij zien elkaar eens per 100 jaar, maar het voelt altijd alsof er nog geen week tussen zat en dat zegt voor mij genoeg.

En tot slot, maar eigenlijk helemaal bovenaan: familie
Elly en Peter, bedankt voor jullie warme onthaal en jullie steun en liefde aan Rick en mij. Opa, ik waardeer de oprechte interesse in mijn onderzoek en opleiding enorm. Ik hoop dat ik net zo vitaal, zelfstandig en bij de tijd oud mag worden.
Ien, je bent zoveel meer dan gewoon een tante.
Daan en Rik, ik heb geprobeerd het hier op te schrijven, maar het is niet uit te leggen hoeveel jullie voor mij betekenen.
Pap, jouw (soms disproportionele) trots maakt me gelukkig. Katrin, bedankt dat je in paps leven bent gestapt.
Mam, dankzij jou ben ik waar ik ben en wie ik ben. Je gunt me niet alleen de wereld, je hebt er ook altijd alles aan gedaan om hem binnen bereik te brengen.
Pluk, je bent de liefste thuiswerk-collega. Jouw onvoorwaardelijke liefde is geheel wederzijds.
Rick, er zijn niet genoeg woorden. Je laat me zijn wie ik ben, maar voegt daar tegelijkertijd zoveel aan toe.
Kees, allerliefste kleine Kees, ik houd onbeschrijfelijk veel van jou. Je hebt mijn perspectief veranderd. Op alles en voor altijd.