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PERIODONTAL DISEASE AND COGNITIVE DISORDERS

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PERIODONTAL DISEASE AND COGNITIVE DISORDERS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To all those who suffer from dementia

POPULAR SCIENCE SUMMARY OF THE THESIS

Dementia is a syndrome with many causes, the most common of which is Alzheimer´s disease (AD). Approximately 50 million people globally have dementia, which has been projected to increase with a changing population structure, mainly in low- and middle-income countries. The existing treatment for AD is effective against some of the symptoms and do not affect the ongoing disease processes in the brain. Another way to manage the disease is to identify risk factors and intervene in these factors. This is called prevention.

Since the late 1980s, studies have shown that diseases of the mouth, especially periodontitis, seem to be more common among people with different kinds of diseases. It has been suggested that periodontitis, through the dissemination of bacteria and inflammatory mediators, and tooth loss can increase the risk of other diseases.

This thesis explores how periodontal disease, tooth loss, and cognitive dysfunction interact. We used different types of epidemiological studies to learn how these diseases relate to each other. Our initial findings suggested that living with cognitive dysfunction can negatively affect an individual's oral health status. We found that signs of periodontitis were more common among individuals with cognitive dysfunction. Exploring the oral microbes among those with cognitive dysfunction, we found that some bacteria were more common compared to cognitively healthy individuals.

The first studies made measurements at one point, making inferences in the context of potential causation difficult. Did periodontitis cause dementia, or did dementia cause periodontitis? Or can the relationship be explained by common causes? We used Swedish national registers to study whether exposure to periodontitis or tooth loss prior to dementia diagnosis leads to an increased dementia risk. Individuals with periodontitis did not seem to have an increased risk of developing dementia during eight years of follow-up. On the other hand, we demonstrated that having fewer than 10 teeth increased the probability of a dementia diagnosis during follow-up by almost 16%.

Thus, we found differences between oral health status and cognitive dysfunction or dementia. Due to limitations inherent in observational research, we cannot state that periodontitis or tooth loss represent true causes of dementia. Individuals living with dementia may have poorer oral health early in the disease and may need more preventive measures. The results also underline the importance of good dental health throughout life.

ABSTRACT

Periodontitis and tooth loss have been suggested to be putative aetiological risk factors for dementia and cognitive dysfunction. The identification of new dementia risk factors could lead to new preventive strategies for dementia. The aim of this thesis was to explore whether periodontal disease and tooth loss are associated with cognitive dysfunction, with special reference to dementia.

In **paper I**, 154 cases from the Karolinska Memory Clinic at Karolinska University Hospital and 76 cognitively healthy controls from Huddinge municipality were enrolled in a case-control study. Cases comprised individuals diagnosed with Alzheimer´s disease (AD), mild cognitive impairment (MCI), or subjective cognitive decline (SCD). All participants underwent dental examinations that included panoramic imaging. The primary exposure was radiologically verified marginal alveolar bone loss (MABL). Generalised MABL was found to be more prevalent among cases than controls, especially for the AD subgroup. No between-group differences were found for localised MABL. In addition, cases had an overall poorer oral health status than controls.

Paper II explored the subgingival microbiota among AD, MCI and SCD participants and controls from **paper I**. Using 16S rRNA gene sequencing, the compositions of the microbial communities were compared across study groups. Only relatively subtle differences were seen. As signs of periodontitis were more common among the cases than the controls, it was difficult to determine whether there would have been actual differences had the periodontitis distributions been the same. In periodontitis-adjusted models, we demonstrated that the bacterium *Prevotella oulorum* was present at a higher abundance among cases than controls and that the bacterium *Rothia aeria* was less abundant.

In **paper III**, we investigated the dementia incidence in a cohort with or without periodontal disease at baseline. Data were retrieved from several national registries in Sweden, such as the Swedish Quality Registry for Caries and Periodontal Diseases and the Swedish Dementia Registry. During the average eight years of follow-up, the incidence of dementia was shown to be similar in the two groups. No association was evident between periodontal disease and dementia in confounder-adjusted regression models.

Paper IV was a cohort study using data from Swedish national registries to investigate whether tooth loss is associated with the incidence of dementia. Two exposure groups were defined at the start of the observation period and followed for up to nine years. Severe tooth loss (STL) was contrasted with a reference group without the index condition. The dementia incidence was higher in the group with STL than in the reference group. This finding was found to be robust in sensitivity analyses and the confounder-adjusted models.

In conclusion, the results in this thesis show the complexity of interactions between dental disease and cognitive dysfunction. Among participants with cognitive dysfunction, signs of generalised MABL were more prevalent compared to controls. Differences in the subgingival microbiota were seen, suggesting that cognitive dysfunction was associated with periodontal

disease. In a longitudinal study, periodontal disease was not associated with an increased risk of dementia. In contrast, having severe tooth loss was associated with an increased incidence of dementia. Thus, severe tooth loss may serve as a marker of dementia risk.

LIST OF SCIENTIFIC PAPERS

This thesis is based on four papers, which will be referred to by their Roman numerals throughout the text.

I. **Holmer J**, Eriksdotter M, Schultzberg M, Pussinen PJ, Buhlin K. Association between periodontitis and risk of Alzheimer′s disease, mild cognitive impairment and subjective cognitive decline: A case-control study. *Journal of Clinical Periodontology. 2018;45(11):1287–98.*

II. **Holmer J**, Aho V, Eriksdotter M, Paulin L, Pietiäinen M, Auvinen P, Schultzberg M, Pussinen PJ, Buhlin K. Subgingival microbiota in a population with and without cognitive dysfunction. *Journal of Oral Microbiology. 2021;13(1).*

III. **Holmer J**, Eriksdotter M, Häbel H, Hed Myrberg I, Jonsson A, Pussinen PJ Garcia-Ptacek S, Jansson L, Sandborgh-Englund G, Buhlin K. Periodontal conditions and incident dementia: a nationwide Swedish cohort study. *Journal of Periodontology. 2022;1-9.*

IV. **Holmer J**, Eriksdotter M, Häbel H, Jansson L, Pussinen PJ Garcia-Ptacek S, Sandborgh-Englund G, Buhlin K. Severe tooth loss and dementia in a Swedish population-based cohort: a registry-based study. *In manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

16S rRNA	16S ribosomal RNA gene
Aβ	Amyloid β or amyloid β 42
AD	Alzheimer´s disease
aHR	Adjusted hazard ratio
APOE4	Apolipoprotein E4 genotype
ARIC	The Atherosclerosis Risk in Communities Study
ATC	Anatomical Therapeutic Chemical
aOR	Adjusted odds ratio
CAL	Clinical attachment loss
CI	Confidence interval
CCI	Charlson Comorbidity Index
CDC/AAP	Centers for Disease Control and Prevention/American Academy of Periodontology
CSF	Cerebrospinal fluid
DAG	Directed acyclic graph
DHR	Swedish Dental Health Register
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DPPD	Deep probing pocket depth
EI	Eichner index
EPS	Extracellular polymeric substances
eHOMD	Expanded Human Oral Microbiome Database
FAD	Familial early-onset Alzheimer´s disease
LISA	Swedish Longitudinal Integrated Database for Health Insurance and Labour Market Studies
LPS	Lipopolysaccharide
HR	Hazard ratio
ICD-9	International Classification of Diseases version 9
ICD-10	International Classification of Diseases version 10
ICSP	International Committee on Systematics of Prokaryotes
MABL	Marginal alveolar bone loss

MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MMI	Mild memory impairment
NBHW	Swedish National Board of Health and Welfare
NHIRD	Taiwan's National Health Insurance Research Database
NIA-AA	The National Institute on Aging—Alzheimer's Association
NPR	Swedish National Patient Register
OR	Odds ratio
PI	Panoramic imaging
PIN	Personal identity number
PPC	Periodontal profile class
PPD	Probing pocket depth
RNA	Ribonucleic acid
SCD	Subjective cognitive decline
SKaPa	Swedish Quality Registry for Caries and Periodontal Diseases
SR	Systematic review
STL	Severe tooth loss
SveDem	Swedish Dementia Registry
VaD	Vascular dementia
VCI	Vascular cognitive impairment
WGS	Whole-genome sequencing

1 INTRODUCTION

Dementia is a syndrome that encompasses several different disorders, the most prevalent of which is Alzheimer´s disease (AD).[1] There is no effective treatment against AD, though much research effort has been invested in pharmacological developments. In the absence of an effective treatment strategy, another focus has been on identifying modifiable risk factors. Identifying and establishing aetiological risk factors is pivotal to primary prevention. Proper implementation of primary preventive interventions has been projected to reduce the incidence of dementia, especially as we still cannot account for a large part of dementia risk.[2]

Oral diseases, mainly dental caries and periodontitis, are highly prevalent globally. Even though oral health has improved dramatically in recent decades, oral disease still presents a tremendous burden, also in Western countries. Recent estimates show that dental caries is the most common non-communicable disease, and periodontitis is the sixth most common.[3,4] Both diseases are associated with microbial dysbiosis and a complex interplay with the host response.[5,6] Effective treatment for both diseases aims to eliminate or reduce dysbiotic dental biofilms. Periodontitis is a slowly progressive inflammatory disease of the tooth-supporting tissues, leading to true loss of tissue attachment. There is a risk for progression and, ultimately, tooth loss if left untreated.[7] The reduction of dysbiotic dental biofilms in periodontitis, which forms the biological basis of treatment, leads to the resolution of periodontal inflammation and eventually stops progressive tissue destruction.

Since the 1980s, there has been much interest in what has been termed "periodontal medicine", a subdiscipline of periodontology that investigates periodontal-systemic disease interactions.[8] The aim has been to explore periodontitis as a potential risk factor for a wide array of diseases and conditions, among which cardiovascular disease has gained the most traction. In addition, diabetes mellitus, adverse pregnancy outcomes, renal disease, rheumatoid arthritis, and cancer have all been associated with periodontitis.[9] No causal relationships have been established; thus, periodontitis has not been proven to contribute to the risk of any of the studied outcomes. To a large extent, ethical considerations preclude the use of experimental/interventional studies, a study design that has the inherent property of effectively dealing with biases that often arise in observational studies. Even if, from an ethical perspective, it would be possible to conduct an intervention study on periodontitis and a systemic disease outcome, it could prove difficult due to the chronic nature of periodontitis.

The focus of this thesis is the exploration and investigation of dental diseases, especially periodontitis and tooth loss, in relation to cognitive dysfunction, with an emphasis on dementia. The studies are observational and use different epidemiological study designs to assess the exposure-outcome relationship between periodontitis, tooth loss, cognitive dysfunction, and dementia. As will be discussed in the thesis, it is challenging to demonstrate a causal relationship using observational studies, but the first step is always identifying putative risk factors (associations).

The studies in this thesis are based on researcher-generated data, analysis of the subgingival microbiota, and the use of the vast nationwide population-based registries in Sweden. Different approaches were used in an effort to increase our knowledge of periodontitis-tooth loss-dementia interactions that, if proven causal in the future, could constitute a target for primary prevention to reduce the burden of dementia and provide a new strategy for dementia management and prevention.

2 LITERATURE REVIEW

This chapter should be read as an introduction to the research field. It does not follow systematic review (SR) methods.[10] The literature has been chosen by the author to reflect the current status of the field (narrative literature review).

2.1 HISTORICAL PERSPECTIVES

Oral diseases were mentioned as a cause of systemic effects in the historical literature, even in preserved documents from ancient civilizations.[11] In a more contemporary context, the so-called focal infection theory was practised in the early twentieth century in the form of tonsillectomies and tooth extractions to alleviate systemic disease. However, the theory was controversial since its introduction.

The period that was later called "an orgy of extractions" (i.e., around 1910 to 1940) began decades earlier with Willoughby D. Miller.[12] Miller published a series of articles in the late 1800s and introduced the theory that infections in the oral cavity can cause other diseases.[13] The ideas were later picked up by William Hunter, who began to question the treatment of severely damaged teeth and opted for tooth extractions instead.[14] Hunter made a clear and famous statement on his opinions:[15]

"*Gold fillings, gold caps, gold bridges, gold crowns, fixed dentures, built in, on, and around diseased teeth, form a veritable mausoleum of gold over a mass of sepsis to which there is no parallel in the whole realm of medicine or surgery. The whole constitutes a perfect gold trap of sepsis of which the patient is proud and which no persuasion will induce him to part with.*"

These claims lacked scientific support, but the ideas had many prominent advocates.[11] The consequence was the radical removal of diseased teeth with the intention to treat systemic disease.[16–18] It soon became evident that this eminence-based practice did not lead to any health benefits for the patients.[19,20] In light of this, Reiman and Havens published a literature review of tooth extraction as a treatment option for systemic disease and concluded:[21]

"(a) The theory of focal infection, in the sense of the term used here, has not been proved, (b) the infectious agents involved are unknown, (c) large groups of persons whose tonsils are present are no worse than those whose tonsils are out, (d) patients whose teeth or tonsils are removed often continue to suffer from the original disease for which they were removed, (e) beneficial effects can seldom be ascribed to surgical procedures alone, (f) beneficial effects which occasionally occur after surgical measures are often outweighed by harmful effects or no effect at all, and (g) many suggestive foci of infection heal after recovery from systemic disease, or when the general health is improved with hygienic and dietary measures."

Even though the practices around focal infection fell out of favour, the ideas persisted. In subsequent years, interest in focal infection research was almost non-existent. It would change in 1989 when a paper with a modern study design was published in BMJ and showed a relationship between poor oral health and myocardial infarction.[22] The study resulted in

new research interest in dental infections as a putative risk factor for systemic disease, and many studies have been published since. Despite numerous studies, causal relationships have not yet been proven.

2.2 AN OUTLINE OF ORAL MICROBIOLOGY

2.2.1 Taxonomy and methods to characterize the microbiota

Over the years, several systems for classifying life have been proposed; the latest is a consensus classification published in 2015.[23] A previous classification scheme used three primary ranks to divide Earth's cellular life into Eukarya, Bacteria, and Archaea.[24] In the most recent classification scheme, life is divided into two superkingdoms: Prokaryota (Archaea and Bacteria) and Eukaryota (with the kingdoms of Protozoa, Chromista, Fungi, Plantae, and Animalia).[23] Bacteria are ordered in the following taxonomic ranks: Phylum, class, order, family, genus, and species.[25]

Bacteriological nomenclature is administered by the International Code of Nomenclature of Prokaryotes (ICNP) kept by the International Committee on Systematics of Prokaryotes (ICSP).[26] New proposals for bacterial names are reviewed by the ICSP in relation to the ICNP (latest revision 2008). If the name meets the criteria, it is officially introduced in the *International Journal of Systematic and Evolutionary Microbiology*. Presently, polyphasic taxonomy, i.e., both genetic and phenotypic techniques, is used to classify bacteria. Before the development of genetic sequencing for the taxonomic classification of bacteria in bacteriology, cultivation was the method of choice and is still used to describe the phenotype. The phenotypic description is determined after cultivation and mainly includes characterization of the morphology, the response to Gram staining, and the determination of the biochemical profile.[25]

Culture-independent high throughput sequencing can be divided into two main approaches: sequencing of the 16S ribosomal RNA (16S rRNA) gene and whole-genome sequencing (WGS).[27] The gold standard molecular phylogenetic and taxonomic marker for bacterial identification and classification is 16S rRNA sequencing. It was introduced by Carl Woese in 1977 as a way to study phylogenetic relationships.[28] 16S rRNA forms a part of the prokaryotic small ribosomal subunit (30S subunit).[29] The 16S rRNA gene is evolutionarily conserved and, in combination with hypervariable regions, used to identify bacteria and profile large bacterial communities.[25] The hypervariable regions are species-specific and can be used for classification, whereas the conservative regions are used to bind primers that enable sequencing. The methods have been refined over the years, making high throughput sequencing techniques very accessible and relatively low-cost over the last few years. WGS involves massive parallel sequencing of the whole genome for phylogenetic identification.[30] The procedure is computationally intensive and requires expertise in bioinformatics for data processing.

2.2.2 The oral microbiota

The expanded Human Oral Microbiome Database (eHOMD) is a curated phylogeny-based database of bacterial species in the oral cavity, pharynx, nasal passages, sinuses, and oesophagus.[31,32] In the latest update, the eHOMD included 775 microbial species (30% uncultivated phylotypes). Dewhirst et al. published an analysis of the Human Oral Microbiome Database (HOMD version 10) that identified the phylogenetic distribution of oral taxa.[33] They found that the most prevalent bacterial phylum was Firmicutes (representing 36.7% of the total taxa). The continued distribution at the phylum level was as follows: Bacteroidetes (17.3%), Proteobacteria (17.1%), Actinobacteria (11.6%), Spirochaetes (7.9%), Fusobacteria (5.2%), Saccharibacteria or TM7 (1.9%), Synergistetes (1.6%), Chlamydiae (0.2%), Chloroflexi (0.2%), and SR1 (0.2%). Zhou et al. conducted a study using 16s rRNA-based data collected from the Human Microbiome Project to investigate the pattern of biogeographic composition among healthy individuals.[34] The biodiversity at 22 different sites was analysed. They found that the oral habitat had the highest alpha diversity compared to other body compartments with bacterial habitats, and the oral microbiota showed high temporal stability. The resident oral microbiota can be divided in different ways, but frequently into the core (or indigenous) and variable microbiota. The variable microbiota consists of microorganisms determined by the environment, such as medical status and dietary patterns.[25] The oral microbiota exhibits diversity between different climate zones, and its composition is associated with lifestyle factors, such as smoking.[35,36] Many different bacteria will transiently pass through the oral compartment, as it is the first entry into the gastrointestinal tract. However, they are usually not adapted to the local oral environment and cannot colonize the oral cavity. On the other hand, the resident microbiota is well adapted and, together with the saliva and mucous membrane barrier, forms an important part of the defence of the oral cavity.

Bacteria are probably the most studied part of the oral microbiota, but the oral cavity harbours several other microbes, including viruses, protozoa, fungi, and archaea. The oral virome is far from fully understood and less investigated than its bacterial counterpart. In a recent study on human saliva samples analysed by single-virus genomics and metagenomics, viral isolates could be grouped into approximately 200 “major clusters”.[37] The authors concluded that their data could not support the existence of a simple core, salivary, viral microbiome. Regarding the presence of protozoon species in the oral cavity, only two nonpathogenic saprophytes have been identified: *Entamoeba gingivalis* and *Trichomonas tenax*.[38] The oral mycobiome was recently characterized among 20 healthy participants using pyrosequencing.[39] The oral mycobiome contained 85 fungal genera. The most frequent fungal isolates were Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium, and Cryptococcus. The prokaryotic Archea constitute a small part of the microbiome. *Methanobrevibacter oralis* is often found, whereas *Methanobacterium curvum*/*congolense* and *Methanosarcina mazeii* are more sparse findings.[40]

2.2.3 The oral environment and formation of dental biofilms

The oral cavity provides favourable conditions for microbial colonization and growth. It offers various physicochemical environments and microbial habitats, compromising the hard dental tissues, saliva, gingival crevices, and mucosa among other compartments.[25] The diverse intraoral habitats harbour specialised microbial communities well-adapted to the local environment and conditions.[41] The tooth surface is non-shedding, which creates good conditions for the establishment of complex microbial communities (dental biofilms) compared to mucosal surfaces. Below is a brief description of dental biofilm formation, which forms the biological basis for the two most prevalent oral diseases: dental caries and periodontitis.

Biofilms are omnipresent and have been defined as: "Aggregates of microorganisms in which cells are frequently embedded in a self-produced matrix of extracellular polymeric substances (EPS) that are adherent to each other and/or a surface."[42] Biofilms can tolerate diverse environmental challenges, ranging from Archaeal biofilms found in deep-sea hydrothermal vents and life in the deep terrestrial biosphere to the bacterial biofilms in the saliva-coated oral cavity.[43]

The dental biofilm develops on the tooth surface and is comprised of microorganisms (mainly bacteria) embedded in a matrix of EPS. The dental biofilm follows a specific order of events for its formation and development.[44,45] Initially, the pellicle, a film derived from saliva, forms on the tooth surface. The next step is a reversible attachment of planktonic bacteria to the pellicle through non-specific forces and receptors. The attachment becomes permanent via adhesins after initial bacterial adhesion to the salivary pellicle. Their nature and structure differ depending on bacteria, but they are usually composed of fimbriae or fibrils. The first bacteria to attach are called primary or early colonizers represented by mitis group streptococci. The primary colonizers facilitate the attachment of secondary or late colonizers.[46] Within days of dental biofilm formation, the next stage of biofilm formation, maturation, commences.[25,44] The main culprits of this stage are increasing biodiversity and biomass.

The well-developed biofilm functions with communication through quorum sensing, production and sharing of nutrition, matrix production, etc. The structure, composition, and stability of the biofilm make it a challenging target for the immune response and drugs. Mechanical manipulation is often considered the most effective way to remove a biofilm.

2.3 PERIODONTITIS

In a recent consensus report, the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, periodontitis was defined as "a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus."[47] The global prevalence of severe periodontitis has been estimated to be 11%.[4] In Sweden, the prevalence of advanced periodontitis has not changed much over time, whereas mild or moderate forms have decreased since the 1970s.[48] The endpoint of periodontitis is tooth loss. In addition, periodontitis is associated with lower quality of life.[49]

Clinical findings that form the basis for a diagnosis of periodontitis have been described in the literature and comprise true clinical attachment loss (CAL), gingival inflammation, deep periodontal pockets, and loss of marginal alveolar bone.[50] A new classification system that defines both periodontal and peri-implant diseases has recently been introduced.[51] The prior main forms of periodontitis (i.e., chronic and aggressive periodontitis) have been re-grouped under the category "periodontitis" due to overlap and no clear pathological distinction between the two former diagnostic entities. Periodontitis is classified based on a stage and grading system. Stage determinations include assessment of severity (CAL, radiographic bone loss, and tooth loss), complexity, extent, and distribution. The grading assessment reflects prognostic factors, such as rate of progression (both "direct" using longitudinal data and "indirect" based on the per cent bone loss/age and case phenotype) and grade modifiers (smoking and diabetes status). No biomarkers are yet available for the diagnosis of periodontitis.

Periodontitis is not caused by a single microorganism but is the consequence of a complex interplay among dysbiosis, modifying factors, and host response mechanisms involving innate and adaptive immunity.[52] The exact mechanisms and determinants that contribute to periodontitis susceptibility and development are still not fully understood, but subgingival dysbiosis, smoking, and diabetes are considered established risk factors for periodontitis.[6] Even though subgingival dysbiosis is considered a risk factor, it has not yet been proven if dysbiosis is a consequence of periodontitis or the other way around. Inflammation can drive the microbial composition towards dysbiosis.[53] In a cohort study it was shown that carriage of *Aggregatibacter actinomycetemcomitans* preceded periodontitis development.[54] Regardless, a necessary cause is the presence of dental biofilm, and indirect evidence is that periodontitis can be treated effectively with interventions targeting the subgingival biofilm.[55] Smoking causes detrimental effects on immune cells.[9] In addition, smokers have a more diverse microbiota than non-smokers.[36] It is unclear which effect of smoking is the most influential in the aetiology and pathogenesis of periodontitis. Diabetes has also been demonstrated to impact the microbial composition in the subgingival microbiota.[56]

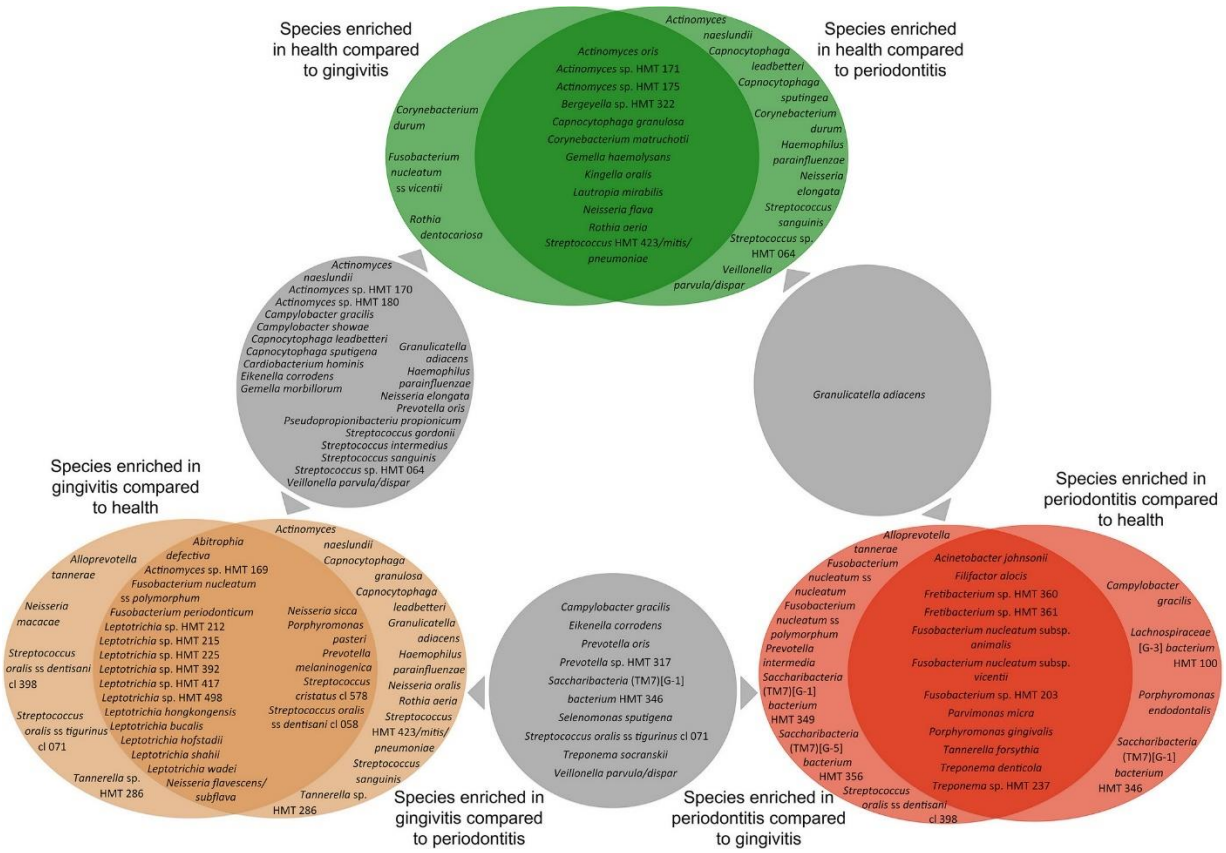

Figure 1. Overview of microbial shifts in periodontal health, gingivitis, and periodontitis.[57] Reprinted with permission from Wiley.

A balance between the oral microbiota and host factors preserves periodontal health. This state has been called microbial homeostasis.[45,58] If the balance is disturbed, consistent with the ecological plaque hypothesis (or more recently, the polymicrobial synergy and dysbiosis hypothesis), periodontitis onset can ensue.[53,59] Recently, the microbial compositional shifts associated with periodontitis have been described and shows that periodontitis has a unique microbial profile (Figure 1).[57] In the subgingival microbiota, periodontitis leads to a higher alpha-diversity compared to periodontal health. Thus, an increase in bacterial richness but a decrease in evenness. The shift from periodontal health to periodontitis is associated with an increased abundance of gram-negative anaerobic taxa. Several bacteria has been associated with periodontitis (the red complex) in classical studies based on cultivation methods.[60] Findings that have been confirmed in recent studies with sequencing techniques.[61] The typical periodontitis-associated taxa (or periopathogens) are *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tanerella forsythia*, *Treponema denticola* and *Prevotella intermedia*. They are all enriched in periodontitis, while the abundance of *Streptococcus* and *Rothia* species decreases. Although this bacteria cluster probably play a key role in periodontitis aetiology and pathogenesis, the microbial compositional shifts and interactions are more complicated than previously thought.

The development of gingival inflammation and periodontitis was described in detail in a classic study by Page and Schroeder.[62] The authors classified periodontal lesions according to four different stages: initial, early, established, and advanced. As pointed out by

Hajishengallis and Korostoff in a recent review, even though our understanding of immunology and microbiology have increased drastically with technological advancements, their classification still holds to a large degree.[63] The initial lesion occurs after a few days of undisturbed dental biofilm accumulation and elicits a characteristic inflammatory response in the connective tissue. As the biofilm accumulates and undergoes maturation, the connective tissue lesion progresses. If disturbances occur in the equilibrium between the host´s defence and the dental biofilm-associated microbiota, it could lead to the development of periodontitis. The most distinctive histopathological alteration in periodontitis is loss of attachment. Though neutrophil granulocytes dominate the initial and early lesions, the distribution of cells in the more advanced lesions are dominated by B cells and plasma cells.[45]

2.4 TOOTH LOSS

Tooth loss is a common consequence of dental disease. In a recent systematic review, the investigators found that dental caries is the most often reported indication for tooth extraction, followed by periodontitis and apical periodontitis.[64] The determinants of tooth loss are complex, and a broader perspective is often needed when investigating the causes and distribution of tooth loss. For example, socioeconomic status is strongly associated with tooth loss.[65] The pattern of tooth loss is also associated with birth cohort.[66]

Oral health has improved in the Western world over the last few decades, and the prevalence of edentulism has declined. In a global context, the prevalence of severe tooth loss (STL) was 2.4% and is decreasing.[67] In a Swedish context, population-based data can be found in national health registers. Comparing 2010 to 2020, it is evident that there is a trend towards an increasing number of teeth in all age groups.[68] For example, the number of remaining teeth among those aged >80 years has increased since 2010 by almost 4; in 2020, the mean number of teeth was estimated to be 21.5. There is also researcher-generated prevalence data available on oral health in Sweden. Since the 1970s, repeated cross-sectional studies have monitored trends in oral health. In the latest investigation, for those aged 70 in 1973, the number of existing teeth was 13.3, which increased to 22.5 in 2013.[69] Despite the declining trend, severe tooth loss is still widespread and can lead to impaired masticatory function that can affect nutritional intake and possibly have psychosocial consequences.[70,71]

2.5 COGNITIVE DISORDERS AND COGNITIVE DYSFUNCTION

Cognitive disorders involve disorders that lead to impaired cognitive functions, typically involving several cognitive domains (e.g., memory, perception, problem-solving, language).[72] The most common form of cognitive disorder is dementia or major neurocognitive disorder, as described in the Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5). Dementia encompasses several disorders, of which AD is the most common.[1] In this thesis, the term “cognitive dysfunction” is used to collectively designate AD, mild cognitive impairment (MCI), and subjective cognitive decline (SCD).

An estimated 50 million people worldwide live with dementia.[73] Thus, dementia is prevalent and, with an ageing global population, the number of persons living with dementia is

projected to increase and put economic pressure on our welfare systems.[74–77] Currently, no effective treatment exists for the most prevalent dementia disorders. A brief discussion concerning cognitive disorders or conditions related to cognitive dysfunction relevant to this thesis follows.

2.5.1 Alzheimer´s disease

AD is the most prevalent type of dementia, representing approximately 60% of cases.[78] The disease was originally described by Alois Alzheimer in 1906 and later named Alzheimer´s disease by Emil Kraepelin in 1910.[79] The clinical manifestations of AD vary depending on disease severity.[1] Early in the disease trajectory, typical memory impairment is evident, with dyspraxia, dysphasia, and signs of impaired visuospatial ability. With disease progression, the symptoms worsen and, it becomes increasingly difficult to work and carry out the normal activities of daily life.[72]

AD is a multifactorial disease. The two most profound risk factors are age and carriage of the APOE ε4 allele (APOE4).[1] A recent report estimated that 12 modifiable putative risk factors could reduce dementia cases (including AD) up to 40%.[80] Different risk factors were deemed more important at different time points in life. Higher education was shown to be protective for dementia in early life. In midlife, hearing loss and traumatic brain injury were the most influential risk factors, whereas smoking, depression, social isolation, and physical inactivity were estimated to be the most critical factors in late life. This approach to risk factor identification (e.g., risk factors have different effects depending on when the factor is measured during a person's life span) has been called life course epidemiology and is probably also applicable to oral-dementia interactions.[81,82] AD has a long preclinical phase, whose various pathobiological steps have been described.[83] The length of the preclinical phase varies and recent estimates have shown that among 70-year-olds it can last for 10 years.[84]

The characteristic histopathological features of AD are an accumulation of plaques and tangles in the brain parenchyma and neurodegeneration. The plaques and tangles are composed of amyloid β (Aβ) and tau protein, respectively.[85] It is still not fully understood whether Aβ and tau cause AD.[86] The most substantial evidence for a causal effect of Aβ originates from studies of familial early-onset Alzheimer´s disease (FAD).[87] Small and Duff suggested a "dual pathway hypothesis" that introduces the possible complex interplay between Aβ and hyperphosphorylated tau in AD causality.[88] An inflammatory component of AD has been known for several decades. Early evidence comes from pathological studies of the AD brain showing activation of microglia and astrocytes and an increase in inflammatory markers, including pro-inflammatory cytokines and complement factors.[89] Data from recent research support the hypothesis that this inflammation contributes to the progression and severity of AD.[90] In addition, peripheral infectious diseases have been associated with AD.[91] Recently, it has been suggested to prioritise intervention studies on a potential microbial AD etiology.[92]

Several classification systems exist for AD. The National Institute on Aging-Alzheimer's Association (NIA-AA) classification system,[93] intended for use in research, was recently revised.[94] In the new classification, the importance of biological markers is emphasized. In addition, the previous clinical entities (preclinical AD, MCI due to AD, and AD dementia) have been more or less abandoned. Instead, the focus is on AD as a continuum. Another classification system for research is the International Working Group criteria published in 2007, which also employs biomarkers.[95] Other diagnostic criteria exist, such as International Classification of Diseases 10th edition (ICD-10) and DSM-5. There is overlap among the classification systems, and all have limitations.

2.5.2 Vascular dementia

Vascular dementia (VaD) is included in the collective term vascular cognitive impairment (VCI), which encompasses disorders caused by vascular damage in the brain, mainly attributable to ischaemia.[96] VaD has been estimated to constitute 15% of dementia subtypes.[97] This is in line with a recent report indicating that VaD represents 11% of the new dementia diagnoses at Swedish memory clinics.[98] Risk factors identified for VaD include age, sex, low education and social class, smoking, low physical activity, hypertension, and diabetes.[96] The clinical manifestations are highly dependent on which brain regions have been affected by vascular damage.

2.5.3 Mixed dementia

VCI also comprises mixed dementia, which is mixed pathology of both AD and cerebrovascular disease. In a Swedish quality register of cognitive disorders and dementia, mixed dementia was diagnosed in 25% of patients who are newly diagnosed with dementia in specialized health care settings.[98] AD and cerebrovascular disease seem to be closely intertwined in dementia.[99]

2.5.4 Mild cognitive impairment

MCI is not a disease in the classical sense but can be described as a collection of symptoms that indicate a decrease in cognition in the range between normal ageing and early dementia that do not affect daily functioning. There are several underlying causes of MCI and different disease trajectories and subtypes (mainly amnestic and non-amnestic).[100] In some instances, MCI can be reversible.[101] Among those with MCI, the annual dementia conversion rate is 5-10%.[102] Prevalence estimates vary across studies; in a North American study conducted in a study population of approximately 2000 individuals aged 70 to 89 years, the prevalence was 16%.[103] Winblad et al. introduced "general criteria for MCI" in 2004, which have been widely implemented.[104] An individual needs to report impairment of cognitive function and cognitive decline, but with no impact on daily living, and they should not meet diagnostic criteria for dementia.

2.5.5 Subjective cognitive decline

SCD is a condition that was defined by Jessen et al. as a self-perceived decline in cognitive function but with normal findings in neurocognitive tests.[105] Individuals presenting with SCD may have an increased risk for dementia, especially AD.[106] SCD is a heterogeneous group with several different causes; therefore, it is prudent to be careful in the diagnostic process.[107] SCD is not a part of ICD-10, though there is a code for subjective mild cognitive impairment (R41.8A), but the concept has been introduced in the NIA-AA classification system.[94] The SCD prevalence among individuals >60 years old varies from 6% to 53%, depending on the study.[108]

2.6 THE INTERACTION BETWEEN ORAL HEALTH, COGNITIVE DECLINE, AND DEMENTIA

One of the first epidemiological studies reporting an association between dental status and AD was published in 1994.[109] Using a case-control study design, Kondo et al. enrolled 60 cases and 120 gender- and age-matched controls. Even though it was a small study, they found that tooth loss was associated with AD. This work was preceded by animal studies. For example, Gobel and Binck used a cat model to show that extirpation of tooth pulp resulted in degenerative changes in the brain.[110] In a study in rats, all molar teeth were extracted, and the animals given easily chewed food for 135 consecutive weeks.[111] The partially edentulous rats made more radial arm maze errors than control rats.

A growing number of studies have revealed associations between periodontitis, tooth loss, and dementia. The SR is the comprehensive synthesis of existing knowledge and should preferably be performed by specialised methodologists.[10] Several SR and meta-analyses have been published on the relationship between periodontitis and dementia; some have been criticized for not following the strict methodology for SR.[112,113] Actually, the scientific contribution of the vast number of published systematic reviews and meta-analyses, which almost outnumber thoughtful, primary research, has been questioned.[114]

2.6.1 Periodontitis, cognitive decline, and dementia

The relationship between periodontitis and AD was recently outlined in a narrative review focused on longitudinal studies.[115] There is a sparsity of longitudinal studies. The few that have been conducted have a rather short observation period, which is a limitation if we are interested in aetiology and consider the long induction period of AD.[81] Another limitation is that the definition of periodontitis exposure differs across studies, a fact that complicates comparisons. In the next section, we describe a selection of studies that have been important for the field.

In one of the first published longitudinal studies, Kaye et al. conducted a cohort study based on approximately 600 men previously enrolled in the Veterans Affairs Dental Longitudinal Study.[116] Participants underwent physical and cognitive examinations, including periodontal assessments, every three years. Cognitive function was evaluated using the Mini-Mental State Examination (MMSE) and a spatial copying task.[117] The cohort was followed for more than

30 years. It was demonstrated that a high rate of tooth loss and progression of periodontitis were associated with lower MMSE and spatial copying scores during follow-up.

Several published cohort studies investigating the link between periodontitis and dementia have been based on secondary data collected from the same source, Taiwan´s National Health Insurance Research Database (NHIRD).[118–122] The NHIRD is a population-based register providing insurance claims data in Taiwan.[123] The studies are similar by design but have subtle differences in age restrictions, observation periods, and certain exposure and outcome definitions (ICD-10 codes or treatment codes). All studies have shown associations between periodontal disease and dementia, with consistent effect sizes across studies. In 2017, Chen et al. published a cohort study using register-based data collected from NHIRD.[118] They enrolled 9291 individuals with newly diagnosed periodontitis and 18,672 matched controls. After a mean follow-up of 12 years, they found that periodontitis exposure was associated with increased risk of AD with an adjusted hazard ratio (aHR) of 1.71 (95% confidence interval [CI] 1.15 to 2.53). In a more recent study based on NHIRD data, patients with or without periodontitis were followed over time.[121] As in the study by Chen et al., the authors found an association between periodontitis and dementia. They also identified protective factors for dementia among patients with periodontitis. The protective factors were related to current pharmacological treatment (statin and metformin) and prior influenza vaccination. Another recent cohort study based on secondary data collected from the Korean National Health Insurance Service-Health Screening Cohort in South Korea also found an association between chronic periodontitis and all-cause dementia (aHR 1.06 [95% CI 1.01 to 1.11]).[124]

Longitudinal studies have been conducted in other settings. In a Swedish study, the investigators enrolled 715 participants and followed the cohort for six years. Using marginal alveolar bone loss as the exposure measure, they found a confounder-adjusted odds ratio (OR) of 2.2 (95% CI 1.2 to 3.8) for cognitive decline.[125] In contrast, a Japanese study found no association between periodontitis and cognitive dysfunction in a cohort study (n = 2335) with an observation period of five years.[126] The Community Periodontal Index (CPI) was used to define the exposure.[127] CPI code four was contrasted with codes one to three, which showed no association with the outcome mild memory impairment (MMI). Furthermore, two recent studies have been published based on data from the Atherosclerosis Risk in Communities Study (ARIC), a prospective cohort study ongoing since 1989 in the US.[128] The first study, published in 2020, was a cohort study comprising 4559 participants who were followed from 1996-1998 to 2011-2013 (mean follow-up 18 years).[129] At baseline, the participants underwent a periodontal examination and were subsequently classified into different periodontal classification schemes. Associations were found between periodontal disease and incident dementia and MCI, but the associations depended on the type of periodontitis classification system used. The primary exposure measure was the periodontal profile class (PPC) derived from the Periodontal Profile Phenotype System.[130] The dementia incidence was higher among participants with severe PPC compared to those in the no PPC group (confounder-corrected hazard ratio [HR] 1.22 [95% CI 1.01 to 1.47]). Null findings were evident using the periodontitis definitions described in the Centers for Disease Control

and Prevention/American Academy of Periodontology (CDC/AAP) classification.[131] The second study, published in 2021, investigated periodontitis in relation to different markers of VCI and AD.[132] The findings were compatible with no association between periodontitis and brain volume, microhemorrhages, or elevated Aβ levels.

In a recent quasi-experimental study comprising about 600 participants, the investigators showed that periodontal treatment positively affected brain atrophy in AD.[133] A study based on Mendelian randomisation did not show an apparent association between periodontitis and AD.[134]

Thus, the literature is inconsistent about the nature of the association. The studies based on Taiwanese and South Korean insurance claims data exhibit similarities and demonstrate a positive association between periodontitis and dementia. In contrast, as seen in the ARIC data, the US data are somewhat inconclusive.

Studies investigating mechanistic pathways between oral health and dementia are sparse. At present, there are mainly narrative review articles that conceptualize what a plausible mechanism would look like (Figure 2).[135–140] The two main suggested biological pathways are translocation of oral bacteria to the central nervous system or gut (the microbiome–gut–brain axis) and periodontitis as a source of pro-inflammatory mediators that disseminate in the systemic circulation and reach the brain.

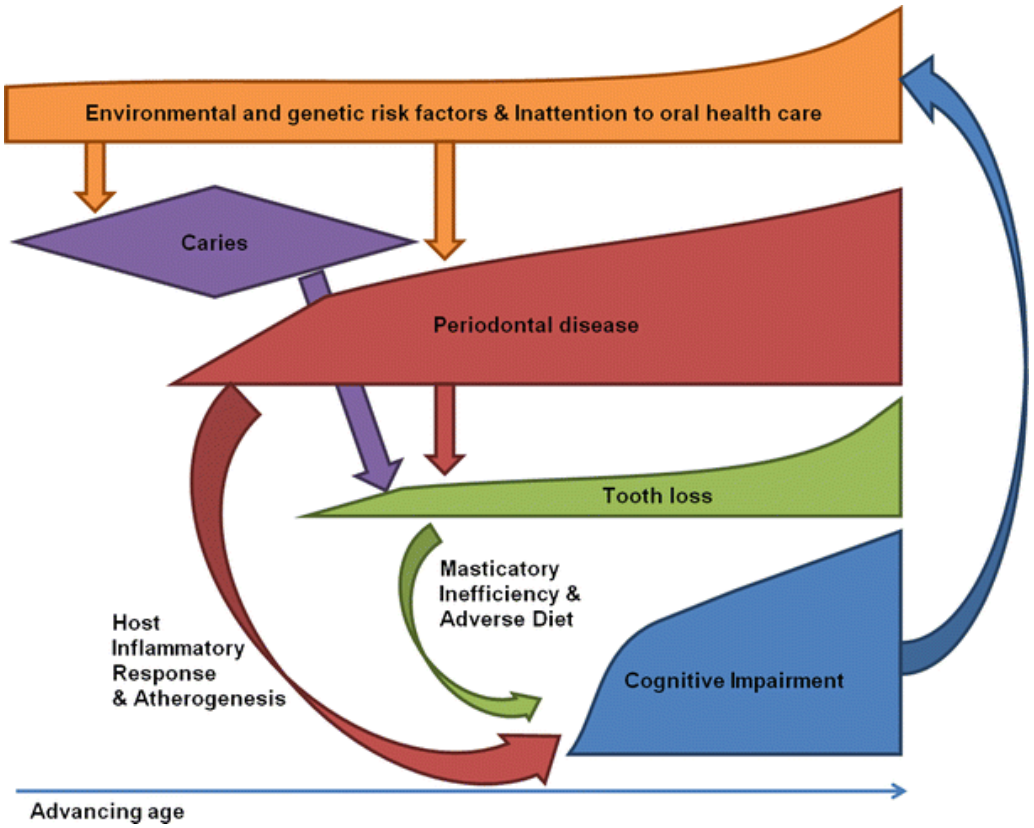

Figure 2. Pathways linking oral health and cognitive impairment.[135] During the life course, a complex interplay among different determinants work together to cause dental caries and periodontitis, which could lead to tooth loss and systemic dissemination of oral bacteria and inflammatory markers, and possibly have a negative effect on cognition. Reprinted with permission from Springer.

The microbiome-gut-brain axis introduces the concept of complex interactions between the gut and central nervous system.[141] A comprehensive review of the field is outside the scope of this thesis, but the gut microbiota has been suggested to constitute an important component in several diseases, as well as neurocognitive disorders.[142]

A small exploratory study obtained postmortem biospecimens from AD and non-AD donors to assess if oral bacteria, primarily oral Treponema, could be detected in the brain.[143] Several Treponema species were more commonly detected in the frontal cortex and trigeminal ganglia of AD donors than non-AD donors using polymerase chain reaction (PCR). The authors also used immunohistochemistry to explore the presence of oral bacteria using species-specific antibodies. *Treponema Pectinovorum* and *Treponema socranskii* were more common in AD donors than non-AD donors. It was also more common to have spirochetes detected in the AD specimens than non-AD specimens. Neither *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* or *Prevotella intermedia* could be detected using PCR.

A common approach is to study inflammatory markers or antibodies to specific bacteria in, for example, plasma or serum. A large cross-sectional study based on the Third National Health and Nutrition Examination Survey (NHANES-III) showed an association between systemic exposure to *Porphyromonas gingivalis* antibody levels in serum and poorer scores on neurocognitive tests.[144] Another cross-sectional study aimed to assess whether individuals with AD had increased plasma levels of TNF-α and antibodies against periodontal bacteria compared to cognitively healthy controls.[145] Although small sample size (n = 34), the authors found that the AD group was associated with increased plasma levels of TNF-α and IgG antibodies against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia*. In a nested case-control study based on the BRAINS (Biologically Resilient Adults in Neurological Studies) program that studies cognitive decline longitudinally, the authors assessed if baseline serum antibodies levels against bacteria associated with periodontitis differed across AD, MCI, and cognitively normal controls.[146] All participants (n = 158) were cognitively normal at baseline. High baseline levels of *Fusobacterium nucleatum* and *Prevotella intermedia* antibodies were associated with AD.

2.6.2 Tooth loss, cognitive decline, and dementia

Tooth loss has been studied extensively as a potential cause of cognitive decline. The suggested biological mechanisms linking tooth loss to dementia or cognitive decline include past oral inflammatory disease (i.e., periodontitis, see section 2.6.1), reduced somatosensory input due to impaired masticatory function, poor nutrition, early life cognitive function, and shared risk factors.[82,147] Since the pioneering study by Kondo et al. in 1994, studies in various settings and with different designs have been published.

In a prospective cohort study in China with a 13-year observation period and sample size of 8153 participants, a lower number of teeth correlated with faster cognitive decline compared to individuals with a higher number of teeth during follow-up.[148] Okamoto et al. aimed to

investigate whether tooth loss is related to MMI and whether this association is influenced by APOE4.[149] This was done using data collected as part of the prospective Fujiwara-kyo study, which is ongoing since 2007 in Japan with a nested case-control study design. The adjusted OR (aOR) associated <9 teeth with MMI was 1.97 (95% CI 1.13 to 3.44). The combination of APOE4+ and having less than nine teeth demonstrated a stronger association, suggesting a synergistic effect. In addition, large-scale studies based on register data have shown associations between tooth loss and dementia.[150,151] A large study from our group based on Swedish registry data has shown an association between tooth loss and dementia.[152] VaD and lower baseline neurocognitive test scores were predictors of tooth loss. The results suggest that impaired cognitive functions may negatively affect oral health.

An alternative way to describe teeth counts is to define the degree of occlusal support, which can be done with the Eichner index (EI).[153] The EI categorizes individuals depending on the number of supportive zones into three main categories (A to C) with subgroups, among which category C has lost all supporting zones. To study whether the loss of posterior occlusal support (POS) is associated with cognitive decline, another Japanese study enrolled approximately 500 individuals aged 79-81 years.[154] The exposure was complete loss of POS, and the outcome was a decrease on a neurocognitive test. The two exposure groups were followed for up to three years. The complete absence of POS was associated with an increased risk of cognitive decline (aOR 1.61 [95% CI 1.03 to 2.49]).

A Swedish study using EI to categorize participants according to their masticatory function at baseline followed a cohort of 544 individuals with a median follow-up of 10 years.[155] The authors demonstrated faster cognitive decline for those in the EI category B and C than category A. The findings indicate that reduced masticatory function was associated with cognitive decline. However, they did not find associations between the different EI categories and incidence of dementia.

2.7 IDENTIFYING CURRENT KNOWLEDGE GAPS

Studies on oral-systemic disease interactions have been conducted since the 1980s, especially on cardiovascular disease. However, the relationship between periodontitis, tooth loss, cognitive decline, and dementia is still in its infancy. The existing literature generally shows consistent positive associations between periodontitis, and cognitive decline or dementia.[115] Few studies show negative findings, which may support an actual relationship (or represent publication bias). Thus, there is a great need for well-designed and well-conducted aetiological studies that longitudinally examine periodontitis as exposure and dementia as an outcome. Due to ethical limitations, it will undoubtedly prove to be a challenging task to conduct classical experimental studies. Nonexperimental studies need to fill this gap. Decisive factors for valid inferences comprise thoughtful study design, long observational periods (due to the chronic nature of periodontitis and dementia), substantial sample size, extensive confounder data, and careful case definitions. In addition, mechanistic studies are needed to explore the biological mechanisms involved.

3 RESEARCH AIMS

3.1 OVERALL AIM

The overarching aim of this thesis was to increase the knowledge of the suggested link between periodontal disease, tooth loss, and cognitive dysfunction with particular reference to dementia.

3.2 SPECIFIC AIMS

- To investigate whether oral health differs among individuals with AD, MCI, or SCD and cognitively healthy controls (**paper I**)

- To explore whether the subgingival microbiota differs between individuals with or without cognitive dysfunction (**paper II**)

- To study the relationships between periodontitis, severe tooth loss, and the incidence of dementia (**papers III** and **IV**)

4 MATERIALS AND METHODS

This chapter outlines study designs and summarises the definitions used in this thesis. Detailed descriptions will be found in the Methods sections of the individual papers.

4.1 STUDY DESIGNS

This thesis consists of four nonexperimental (observational) studies representing two of the most widely used epidemiological study designs: the case-control study (**papers I** and **II**) and the cohort study (**papers III** and **IV**).[156]

Papers I and **II** were based on primary data collection (researcher-generated data), which means that the investigator has decided which variables to measure and in what manner. Data collection are usually conducted through patient examinations, interview, surveys, and/or biospecimen collection.

Papers III and **IV** were based on secondary data collection (pre-existing data). Register-based research often uses data from secondary data sources or a combination of both primary and secondary data sources. Secondary data have often been collected for purposes other than research, such as health surveillance or insurance claims data.

4.1.1 Case-control study on oral health, subgingival microbiota, and cognitive dysfunction

In **paper I**, we conducted a case-control study.[157] It is a common misconception that a case-control study generally yields less valid estimates than a cohort study.[158,159] In fact, the study designs are conceptually very similar, except that the case-control study uses sampling to retrieve the denominator data. Case-control studies include cases from a source population and use a sampling scheme to sample controls from the same source population that produced the cases.[160] Thus, a case-control study would contain the same cases as a cohort study based on the same source population.[161] For valid inference, the control group must represent the distribution of the exposure of interest in the source population and be independent of the exposure. If the case-control study is properly conducted, it can be more efficient than a cohort study due to sampling.[162] In addition, depending on the type of control sampling scheme, a case-control study can provide valid estimates of the incidence rate ratio (density-based sampling) or risk ratio (cumulative sampling or case-cohort sampling).[163]

As mentioned, a case-control study is chosen instead of a cohort study mainly because it is more efficient (time-saving and less expensive). This advantage has some potential limitations by design compared to cohort studies.[156] First, the case-control design does not result in full risk or rate denominator data, only ratio estimates. Second, the case-control study often leads to reduced precision compared to the cohort design because of control sampling; this can be compensated for somewhat if the number of controls per case is increased. Third, difficulties with the sampling could lead to biased estimates.

We enrolled cases (see definitions in section 4.2.1.2) from the Karolinska Memory Clinic at the Karolinska University Hospital in Huddinge between October 2013 and April 2017. Newly diagnosed cases were identified by continuous screening of patient charts during the study period. Thus, the cases could be regarded as incident cases; we aimed to include persons with a first diagnosis no more than one year before the inclusion date. We initiated control enrollment during the late phase of case recruitment. As the control group was frequency-matched for age and sex, we needed an idea of the age and sex distribution among the cases before recruiting controls. The source population was the population of Huddinge municipality because it is the main catchment area for the Karolinska Memory Clinic in Huddinge. The clinic also receives referrals from the Greater Stockholm region, making unbiased control sampling more complicated. Thus, we used a cumulative proxy sampling scheme for our control enrollment and recruited controls from Huddinge municipality by randomly sampling from the population register.

Paper II was based on the same study population as **paper I** but comprised an exploratory cross-sectional analysis of the subgingival samples collected in conjunction with the dental examination. The microbial community compositions were compared across study groups.

4.1.2 Cohort studies on periodontitis, severe tooth loss, and incidence of dementia

For **papers III** and **IV**, we used the cohort study design. The cohort study could be conceptualised as a clinical trial without the assigned intervention and without randomising an exposure across study groups. In cohort studies, a sample is retrieved from the target population and two or more groups (or subcohorts) are defined based on the exposure status. The exposed group usually has the index condition (e.g., a disease, occupation, or other characteristics) that is the subject of investigation in relation to an outcome. The exposed group is contrasted with one or more groups without the exposure (unexposed or reference group). The exposure groups are followed over time until the outcome occurs or when information on outcome occurrence is no longer available (i.e., censoring). The cohort size gets smaller as participants leave the cohort because of the outcome or censoring. At the end of the observation period, the occurrence of the outcome is compared across exposure groups using estimates of incidence.

Papers III and **IV** were cohort studies with fixed cohorts using secondary data (register-based data). In **paper III**, we used matching, restriction, and regression models to control for confounders, whereas in **paper IV** we used restriction and regression models. Data were collected through record linkage among several Swedish nationwide registries, including public authority registers and quality registers. The data linkage was performed by the Swedish National Board of Health and Welfare (NBHW), and data was delivered in a pseudonymised form.

In **paper III**, we identified all individuals aged 40-80 years in 2010 with a registered dental examination in 2010-2012 that included a pocket probing chart in the Swedish Quality

Registry for Caries and Periodontal Diseases (SKaPa). Two groups were defined based on the index condition, periodontitis (see section 4.2.2.8).

In **paper IV**, we identified all individuals aged 60-80 years in 2010 with a registered dental examination in 2010-2012 that included data on teeth counts in the SKaPa. Two exposure levels were explored: with or without the index condition STL (see section 4.2.2.8).

Following identification of exposure groups, the cohorts were followed from the index date (date of dental examination) until outcomes (i.e., all-cause dementia and different types of dementia, see section 4.2.2.9) occurred and the incidence compared across groups. Prevalent dementia cases were excluded from the study cohort before the start of follow-up because they were no longer at risk for dementia.

4.2 DATA SOURCES

4.2.1 Primary data collection

4.2.1.1 Exposure assessment

In **paper I**, the exposures consisted of different dental-related measures collected from a complete dental examination at the Department of Dental Medicine, Karolinska Institutet. Mainly, information was collected on periodontal status, but also earlier restorative dental care, dental caries, and teeth counts. The dental examination also included panoramic imaging (PI) and biological samples.

The primary exposure was marginal alveolar bone level (MABL) assessed by PI. PI has been used extensively in epidemiological studies.[69,164,165] PI is a form of tomography and provides an easily accessible overview of the dentition at a low radiation dose.[166] A diagnostic accuracy study has shown that PI is reliable for periodontitis screening.[167] Even though the measurement of MABL in a panoramic image is cross-sectional information, it could be considered a proxy for longitudinal information because it represents the cumulative periodontitis exposure over a person's life span. Another reason why MABL was chosen as the primary exposure was because it enabled masked analysis, which for practical reasons was not feasible during the clinical examinations. The radiological analysis regarding MABL was done by two observers, masked for group belonging, and a consensus classification for each participant was agreed upon and used in the statistical analysis.

In the absence of a consensus classification regarding MABL, we used the following classification:[164]

- No/mild (loss of supporting bone <1/3 of the root length);
- Localised (loss of supporting bone tissue ≥1/3 the root length in <30% of the teeth);
- Generalised (loss of supporting bone tissue ≥1/3 the root length in ≥30% of the teeth.

Using a questionnaire, we collected personal information and information on medical conditions that also covered important confounders.

4.2.1.2 Outcome assessment

We enrolled cases from the Karolinska Memory Clinic at the Karolinska University Hospital in Huddinge, as described in section 4.1.1. At the Karolinska Memory Clinic, diagnoses are made at a multidisciplinary consensus conference after an extensive work-up and classified using the ICD-10 diagnostic criteria.[168] Furthermore, the following diagnostic criteria should be met:

- For AD, the NIA-AA diagnostic guidelines for dementia due to probable AD.[169] Primarily, patients with MMSE >20 were included.
- For MCI, the Winblad criteria.[104] The criteria state that the patient should be non-demented but not cognitively normal; present with self-reported cognitive decline or from an informant, which also has to be verified through objective neurocognitive tests. Activities of daily living should not be affected.
- For SCD, the pre-MCI SCD criteria.[170] To fulfil the criteria, the patient has to report a self-perceived cognitive decline, show normal performance on cognitive tests and not meet the criteria for MCI, dementia or other diseases that could explain the cognitive decline.

We applied an age restriction (50-80 years) and did not include patients with clinically relevant systemic diseases that could affect participation in the clinical examinations or precluded cerebrospinal fluid collection.

4.2.1.3 Assessment of the subgingival microbiota

In **paper II**, which was a cross-sectional analysis based on the samples collected in **paper I**, we conducted an exploratory study of the composition of the subgingival microbiota among cases and controls. The study design and participant enrolment have already been described.

Subgingival samples were collected after the clinical examination. The deepest or most representative periodontal or peri-implant pocket was identified and sampled in each quadrant. After isolation and removal of the supragingival plaque at the site to be sampled, a sterile curet was inserted to the bottom of the pocket, a sample retrieved with a single pull in the coronal direction, and pooled in tubes with PCR grade water. The tubes were immediately stored in a freezer at −80°C.

The samples were processed for DNA extraction and PCR amplification and the16S rRNA gene (V3-V4 regions) sequenced by Illumina MiSeq according to a published protocol.[171] The microbial profile was determined after primer trimming and quality control. Taxonomic classification and operational taxonomic unit (OTU) assignment were performed using mothur.[172] The main reference database for taxonomic assignment was the eHOMD.

4.2.2 Secondary data collection

4.2.2.1 Register-based research in Sweden

The conditions for register-based research in Sweden and the other Nordic countries are excellent. The availability of national registers with coverage that comprises entire populations creates unique conditions for studying research questions that would otherwise be very difficult to study. Nordic cross-border sharing of data has also been discussed, but mainly legal restraints currently hinder further collaboration.[173]

Decisive factors that have enabled register-based research in Sweden are often attributed to the personal identity number (PIN), tax-funded healthcare, long tradition of keeping high-quality registers (reporting to the registries is often mandatory) and the public´s trust in the scientific community.[174] The Swedish PIN enables record linkage among registers.[175] In Sweden, there are four types of registers: national public authority registers, national quality registers, research-generated data/registers, and biobanks.[176] There are approximately 100 national quality registers.[177]

There are many advantages of using registry data.[178] The data in the registers have been collected continuously and prospectively, often for a long time, and independent of future research. Furthermore, many of the registers have complete population data, which results in substantial sample sizes that give good precision and minimize bias (e.g., selection bias). The pre-existing data also makes using certain study design elements easier, such as matching in cohort studies. There is also an aspect of cost-effectiveness; as the data have already been collected, there is no need for expensive data collection.

Lack of information (notably on exposures, outcomes, and essential confounders) depending on the variables available in the registers and occasionally sparse information on data quality are possibly the main limitations in register-based research. In addition, missing data and left-truncation are common issues.[178]

The use of Swedish national registers for research purposes is regulated by legislation, especially with regard to the protection and integrity of personal data (see section 4.4.2).

A brief description of the registries that provided data for **papers III** and **IV** are presented below.

4.2.2.2 The Swedish Quality Registry for Caries and Periodontal Diseases

The SKaPa is a national quality registry launched in 2008 to monitor dental healthcare in Sweden.[179] The SKaPa collects data directly from patient charts using automated file transfers. Data include demographics, dental status with periodontal pockets and dental caries at tooth/site level, the number of teeth and other basic registrations, including dental care utilization.

In 2018, the SKaPa covered 90% of persons up to 19 years of age and approximately 50% of persons aged >20 years. Data were delivered mainly from public dental care in Sweden

(Folktandvården), but a steadily increasing number of private dental caregivers provide data to the registry. As a national quality register, the data should undergo continuous quality control, outlined in recommendations by the executive committee for national quality registries in Sweden.[180] The SKaPa updates the database retrospectively as new organizations and clinics start to provide data.

In Sweden, a public authority register also provides data on dental health, mainly treatment codes connected to the national dental care subsidy and held by the NBHW, the Swedish Dental Health Register (DHR).[181]

4.2.2.3 The Swedish Dementia Registry

The Swedish Dementia Registry, or the Swedish registry for cognitive/dementia disorders (SveDem), is a national quality registry established in 2007 to improve the diagnostics and management of patients with dementia.[182] In 2020, all memory clinics and 78% of primary care units in Sweden delivered data to the registry. Incident dementia cases (i.e., newly diagnosed) are registered in SveDem and followed up annually. Data on a wide variety of variables are collected. Using a web-based registration, entries comprise demographic and health-related variables, including information on the diagnostic work-up, diagnosis, and treatment.

Eight types of dementia disorders are registered in SveDem: AD, mixed dementia, VaD (including subcortical), dementia with Lewy bodies (DLB), frontotemporal dementia (FTD), Parkinson´s disease dementia (PDD), unspecified dementia, and other dementia types.[98] The ICD-10 is used in Sweden for classifying dementia. For certain dementia disorders, other criteria are used: for DLB the McKeith criteria, for FTL the Lund-Manchester criteria, and PDD the Movement Disorder Society Task Force criteria.[183–185]

The coverage estimates depend on assumptions and the data used for the calculations (e.g., incidence or prevalence, data sources etc.). The incidence of dementia in Sweden has been estimated to be approximately 20,000 to 25,000 cases each year. But there is uncertainty in the estimates.[76,186] Roughly 6400 new registrations were made in SveDem in 2019, giving coverage based on the estimated incidence in the population of 30%.[187] The latest estimated incidence figures, from 2020, have been affected by the coronavirus pandemic and are not representative.[98]

In **papers III** and **IV**, the SveDem was used for case ascertainment.

4.2.2.4 The Swedish National Patient Register

The Swedish National Patient Register (NPR) was established in 1964 and includes two registers, the Swedish National Inpatient Register and the Swedish National Outpatient Register. The registers comprise valid data collected from hospital-based and outpatient specialist care of somatic and psychiatric hospital discharges with very good coverage (complete national coverage since 1987).[188] Primary care data are not a part of the NPR.

Diagnoses have been coded using ICD-10 codes since 1997 (except Skåne, which transitioned to ICD-10 from ICD-9 in 1998). In **papers III** and **IV**, the NPR was used to retrieve data on comorbidities, and for case ascertainment.

4.2.2.5 The Swedish Longitudinal Integrated Database for Health Insurance and Labour Market Studies

The Swedish Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA) is maintained by Statistics Sweden.[189] The LISA includes information on income, education, occupation, migration, and civil status, as well as other socioeconomic information since 1990. The registry retrieves information from several other national public authority registries in Sweden.[190] In **papers III** and **IV**, the LISA was used to retrieve data on education, disposable income, civil status, migration, and geographical regions.

4.2.2.6 The Swedish Prescribed Drug Register

Launched in 2005, this register contains information on dispensed drugs, including dispensed amount, dosage, and expenditure, as well as data on prescriber´s profession and type of practice. Dispensed drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification system. In **papers III** and **IV**, the register was used to identify prevalent and incident dementia.

4.2.2.7 The Swedish Cause of Death Register

The Swedish Cause of Death Register has been available for researchers in Sweden since 1952 and is now maintained by the NBHW.[191] The register contains almost complete data on causes of death among Swedish residents classified according to ICD codes. In **papers III** and **IV**, the register was used to retrieve data on mortality.

4.2.2.8 Exposure assessment

In **paper III**, the exposure data were collected from the SKaPa. An operationalised periodontitis definition was used based on the probing pocket depth (PPD) in the absence of information on CAL or radiologically verified marginal bone loss. At the time of data retrieval from the SKaPa, the database was not designed to distinguish between teeth and dental implants in the periodontal status records. Furthermore, the data were aggregated at the tooth/implant level, not site level. The following exposure groups were defined:

- The exposed group, designated deep periodontal/peri-implant probing pocket depth (DPPD): ≥4 teeth and/or dental implants with PPD ≥6 mm;
- The unexposed group (reference), designated non-DPPD: <4 teeth and/or dental implants with PPD 4 to 5 mm and no PPD ≥6 mm.

In **paper IV**, the information on exposure levels was collected from the SKaPa. The index condition was STL, which was contrasted with a reference group (non-STL). As no accepted definition of STL was available, a definition was chosen based on a systematic review that studied the global prevalence of STL[67]:

- Exposed group, designated STL: 0 to 9 existing teeth;
- Unexposed group (reference), designated non-STL: 10 to 28 existing teeth.

4.2.2.9 Outcome assessment

The cohorts were followed until a first dementia diagnosis, migration, death, or the end of follow-up (31 December 2018), whichever occurred first. The cause of death registry was used to collect mortality data and the LISA to retrieve data on migration.

The primary outcome in **papers III** and **IV** was a first all-cause dementia diagnosis. All-cause dementia diagnosis was defined as a baseline registration in SveDem, a dementia diagnosis recorded in the NPR (ICD-10 codes: F00, F01, F02, F03, G30, and G31 with subgroups), and/or treatment with anti-dementia drugs (ATC code N06D) recorded in the Prescribed Drug Register.

In **paper IV**, we also investigated secondary outcomes. The secondary outcomes comprised the following dementia diagnostic subgroups as defined in SveDem, which also was the data source for the secondary outcomes: AD, VaD, and mixed dementia.

4.2.2.10 Covariate assessment

To take potential confounders into consideration in **papers III** and **IV**, we used data from several national registers. The LISA was used to collect information regarding socioeconomic status and the NPR to collect data on comorbidities. Comorbidities were used as a composite variable using the Charlson/Quan Index (CCI).[192,193] Recently, a new version of the CCI was developed for Swedish register-based studies, but this was not used in **papers III** and **IV**.[194]

4.3 STATISTICAL ANALYSES

4.3.1 Sample characteristics and descriptive statistics

Sample characteristics in **papers I** and **II** were presented as categorical variables, as counts and proportions, in the published tables. Between-group differences were assessed by the chi-squared test. In **paper II**, we also assessed continuous variables, presented as medians with the interquartile range (IQR), and between-group differences were tested by the Mann-Whitney test and Kruskal-Wallis test.

In **papers III** and **IV**, we presented categorical variables as counts and proportions and continuous variables as medians and IQR. Significance testing was not done to assess differences in baseline characteristics. It is a recommendation of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines to avoid using significance testing in descriptive tables.[195]

4.3.2 Microbial community analysis

In **paper II**, bioinformatics analysis was conducted. The R packages Phyloseq and vegan were used for data management and analysis of alpha and beta diversity.[196,197] Alpha diversity

describes the diversity within samples and uses measures of richness or indices that combine richness and evenness (e.g., the Shannon index), whereas beta diversity describes between-sample dissimilarity.[27] The statistical inference for alpha diversity was based on significance testing using Kruskal-Wallis tests, Pearson correlations, Wilcoxon rank-sum tests (post hoc test), and multiple linear regression. For beta diversity analysis, Bray-Curtis dissimilarity was used, and between-group differences were tested using PERMANOVA.

Another way to describe the microbiota is through the analysis of differential abundance. DESeq2 was used to explore differences on the OTU, genera, and family level to determine the differential abundance (i.e., the difference in specific bacterial taxa).[198]

4.3.3 Logistic regression

In **paper I**, binary and multinominal logistic regression models were used to study the exposure-outcome relationship between MABL (and secondary exposures) and cognitive dysfunction. Crude and adjusted ORs (age, sex, marital status, education, smoking, body mass index, and diabetes mellitus) were presented with 95% CIs. Confounder selection was mainly based on subject matter knowledge with support from directed acyclic graphs (DAGs). DAGs are graphical models that can be used when conceptualizing a relationship (usually in a causal context) between an exposure and outcome, and support decisions concerning confounders, colliders, and mediators.[199]

4.3.4 Cox proportional hazard regression model

The Cox proportional hazard regression model, or Cox regression, is a commonly used semi-parametric statistical model in epidemiology for time-to-event outcome analysis on the hazard scale.[200] The Cox regression takes time into account, so each participant contributes time at risk, usually in person-years. The measure of association is the HR, usually accompanied by a measure of precision (95% CI), and is interpreted as an average of the HR over the entire observation period.[201] Notably, the estimate is only valid under the proportional hazards (PH) assumption. Hazard is the instantaneous probability of the outcome; in **papers III** and **IV**, this is represented by dementia risk at a specific time point.

In **paper IV**, HRs for incident dementia were estimated by Cox regression. Crude and adjusted models for age, sex, civil status, disposable income, education, and CCI were provided. Point estimates were presented with 95% CIs. The Schoenfeld residuals was used to test the proportional hazards assumption, which showed that the proportional hazards assumption did not hold for geographic regions, an assumed confounder. Stratified Cox regression analyses across a region were performed with the above covariates in the model. In addition, secondary outcomes (i.e., AD, VaD, and mixed dementia) were separately analysed using Cox regression based on SveDem data in which participants with one of the other secondary outcomes were censored.

Sensitivity analyses were performed to assess how different exposure definitions influenced the relationship between the exposure and the primary outcome measure. The following

sensitivity analyses were performed: exclusion of edentulous participants, the introduction of a washout year, alternative exposure categorizations, and an analysis of the exposure on a continuous scale.

4.3.5 Royston-Parmar flexible parametric survival model

As described above, Cox regression is frequently applied in epidemiological data analysis. The proportional hazards assumption needs to hold for the point estimate (i.e., HR) to be valid. When the hazards are not proportional, another approach needs to be considered to account for the assumption violation. One solution is to choose a more flexible model. In 2002, Royston and Parmar introduced one such model, later designated the Royston-Parmar flexible parametric survival model (RP model), which was originally designed for prediction modelling.[202,203]

In **paper III**, the RP model was applied to explore the exposure-outcome relationship between DPPD and dementia incidence. The point estimates were presented as the exponentiated RP model coefficient, comparable to HRs, with 95% CIs using both crude (unadjusted) and adjusted models. Adjustments were made for age, sex, income, education, civil status, and CCI.

4.4 ETHICAL CONSIDERATIONS

Ethical approval for all studies in this thesis was obtained from the Regional Ethical Review Board in Stockholm (**papers I** and **II**: registration number 2012/652- 31/1 with amendments, and **papers III** and **IV**: registration number 2017/737-31). Informed consent was collected in writing for **papers I** and **II**. Collection of informed consent in writing was not required for **papers III** and **IV** due to Swedish legislation, an aspect that will be discussed later in this chapter.

There are ethical challenges in dementia research, especially when collecting primary data through clinical trials or situations with a risk of adverse events. Individuals with impaired cognitive function may have more difficulty perceiving and understanding the information they are given, including research-related documents and verbal information. A person with early AD may comprehend information and consent to participating in research. However, research suggests that persons with AD relatively early lose the ability to estimate risks and understand written and oral information compared to cognitively healthy individuals.[204]

In **papers I** and **II**, primary data were collected and individuals with dementia physically examined. As we primarily enrolled individuals early in their disease trajectory, the majority were deemed to have autonomous decision-making capacity. In a few instances, a close relative of the participant was provided with all of the participant information, received verbal information about the study, and signed, together with the research participant, an informed consent form. The risks were judged to be low, comparable to undergoing a routine dental examination that includes ionizing radiation and blood sampling. The participants were recruited from the Karolinska Memory Clinic and were offered a free dental examination,

which is likely beneficial for the individual as oral health screening. All study participants and, if applicable, family members were also informed of any clinical or radiological findings.

To prevent the physician from affecting the patient´s decision, the invitation to participate was not made at the Karolinska Memory Clinic. As described in **paper I**, potential participants were first approached with a letter explaining the purpose of the study and what it entails. After a couple of weeks, the participant was contacted by telephone and invited to participate in the study. By following this routine, potential participants were given time to think about whether they wanted to participate, and ask their friends and family. This approach probably reduced stress and the feeling of making forced decisions.

The ethical considerations for **papers I** and **II** were mainly related to informed consent and the difficulty to determine boundaries for consent competence. Clear, consistent, and updated research ethics guidelines for implementing dementia research would be of considerable use to researchers, and patients, and this has been discussed in the literature.[205]

Register-based research in Sweden is regulated by Swedish law. Ludvigsson et al. described the ethical framework that register-based research is based upon in Nordic conditions.[174] All research involving humans in Sweden must undergo ethical review according to the Ethical Review Act (Lag [2003:460] om etikprövning av forskning som avser människor). If approved, studies based on register-data do not need to collect individual informed consent in writing from study participants. Requiring informed consent in writing from all participants for each project would, in practice, make it impossible to conduct register-based research in the way it is conducted today. However, there is an obligation to provide information that data are collected in, for example, a national quality register and that the patient has the right to waive registration or be deregistered.

The main risks with register-based research concern the handling, integrity, and protection of personal data. Therefore, laws exist to regulate the use of personal data and safeguarding personal integrity. The General Data Protection Regulation (GDPR, Dataskyddsförordningen [2016:679]) stipulates guidelines for handling personal information. In Sweden, several complementary laws exist with regulations supporting the GDPR in the context of register-based research and the handling of sensitive personal data. For example, the Health Care Data Register Act (Lag [1998:543] om hälsodataregister) regulates how health registers can be used, and an ordinance (Förordning [2001:707] om patientregister hos Socialstyrelsen) regulates the national patient registers.[188] Medical research and handling of health-related data are also regulated in the Health and Medical Services Act (Hälso- och sjukvårdslag [1982:763]), the Patient Data Act (Patientdatalag [2008:355]), and the Public Access to Information and Secrecy Act (Offentlighets-och sekretesslag [2009:400]).

In **papers III** and **IV**, data were collected from national public authority registers and national quality registers. Data to be delivered for research purposes are often pseudonymised by the NBHW. Following data delivery, a review of the data is conducted and, after three

months, the key linking the serial number and PIN is deleted by the NBHW if the ethical review application does not state that the data should undergo updates. Even though the key is deleted, there is still a risk of breach of confidentiality. For example, it is possible to triangulate an individual by combining different variables. Therefore, it is of paramount importance that all data are stored safely.

5 RESULTS

5.1 PERIODONTAL DISEASE, COGNITIVE DYSFUNCTION, AND DEMENTIA

Paper I comprised 230 participants with 154 cases and 76 control participants. Of the 154 cases, 52 were AD, 51 MCI, and 51 SCD. The participation rate was 65% among cases and 42% among controls.

The primary exposure variable, MABL, was analysed as a categorical variable with three levels: no/mild, localised, or generalised. Generalised MABL was more prevalent among cases (n = 14, 9.2%) than controls (n = 2, 2.6%). In the subgroups, the AD group had the highest prevalence (n = 8, 15.4%), followed by SCD (n = 4, 7.8%) and MCI (n = 2, 4%).

We found an association between generalised MABL exposure and cognitive dysfunction (combined cases group) after adjusting for confounders (aOR 5.8 [95% CI 1.1 to 29.7]). The crude OR for generalised MABL and the combined cases group was 4 (95% CI 0.9 to 18.2). In the subgroup analyses, AD showed the strongest association with generalised MABL (OR 7.4 [95% CI 1.5 to 37.4] and aOR 6 [95% CI 1.0 to 35.1]), together with SCD after adjustments (OR 3.1 [95% CI 0.5 to 18.1] and aOR 12.3 [95% CI 1.7 to 92.2]). The OR and aOR for generalised MABL and the MCI group were 1.7 (95% CI 0.2 to 12.9) and 2.2 (95% CI 0.3 to 17.6), respectively.

The prevalence of localised MABL was similar in the control group (n = 24, 31.6%) and the combined cases group (n = 51, 33.3%). In the diagnostic subgroups, the prevalence was similar (ranging from 29.4% to 38%), and the regression analyses disclosed no apparent association between localised MABL and cognitive dysfunction.

Analysis of secondary exposures showed that cases had an overall poorer oral health than controls, with more caries lesions, higher bleeding scores, and a higher number of pathological PPDs.

In **paper III**, the exposure measurement was a binary variable based on pocket depth data: i.e., with (n = 7992) or without (n = 29,182) DPPD. The two groups were followed for a mean of 7.6 years. At the end of follow-up, 1.7% (n = 137) in the DPPD group had developed dementia and 1.6% (n = 470) in the non-DPPD group. The dementia incidence density rates were also similar across exposure groups: 2.3 per 1000 person-years in the exposed group and 2.1 per 1000 person-years in the non-exposed group.

Kaplan-Meier curves visualize similar conclusions (Figure 3). An association was not apparent using the RP model for all-cause dementia incidence to contrast the two groups. The crude exponentiated RP coefficient estimate was 1.15 (95% CI 0.40 to 3.28), and the adjusted estimate was 1.13 (95% CI 0.39 to 3.24). Sensitivity analyses using a lower cut-off for the exposed group did not change the results.

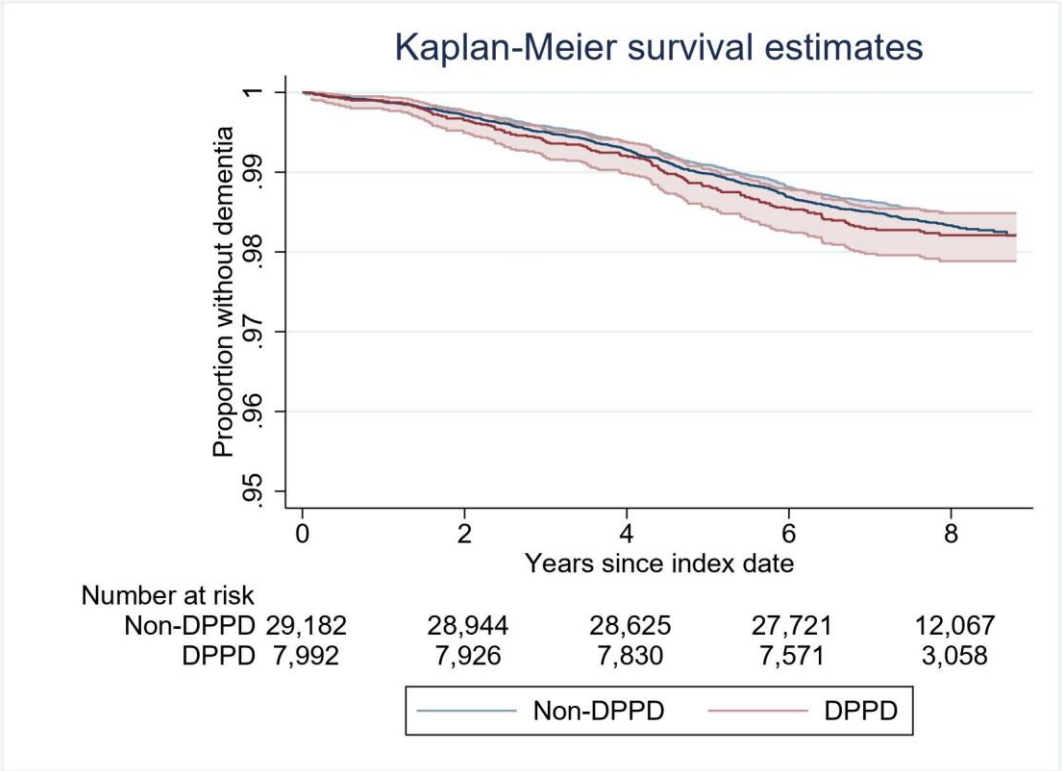

Figure 3. Kaplan-Meier plot of the relationship between deep periodontal pocket depth (DPPD) and dementia. Shaded areas indicate 95% confidence intervals.

5.2 SUBGINGIVAL MICROBIOTA IN INDIVIDUALS WITH COGNITIVE DYSFUNCTION

Paper II explored the subgingival microbiota. Alpha diversity comparisons were performed with observed richness as OTU counts and the Shannon index, which disclosed that the alpha diversity was higher in cases than controls and that the MCI group was the main driver of association. Assessment of beta diversity demonstrated differences between cases and control participants.

Differential abundance comparisons disclosed similarities in the relative abundance of the most common genera (Fusobacterium, Porphyromonas, Capnocytophaga, Treponema, Prevotella, Campylobacter, and Streptococcus) across the study groups. Differential abundance comparisons on the OTU level showed that *Prevotella oulorum* was more common among cases than controls, whereas *Rothia aeria* was present in lower abundance in the cases compared to controls. *Slackia exigua* and *Lachnospiraceae* [G-7] bacteria were more abundant in the AD group than in control participants.

Deep periodontal pockets (≥6 mm) were also associated with higher alpha diversity and associated with several specific OTUs, with an increased abundance of *Porphyromonas gingivalis* and *Prevotella intermedia,* which decreased abundance of *Actinomyces massiliensis*, *Haemophilus parainfluenzae,* and *Streptococcus mutans*.

5.3 TOOTH LOSS AND DEMENTIA

In **papers I**, **II**, and **IV**, teeth counts were investigated in relation to cognitive dysfunction and dementia.

In **paper I**, the number of teeth was collected and analysed cross-sectionally. The median number of teeth was 27 for all cases combined and the control group, and 26, 26, and 27 existing teeth for the AD, MCI, and SCD subgroups, respectively. Fewer than 20 teeth were present in 9.2% (n = 7) of controls and 8.4% (n = 13) of cases; among the subgroups, 11.5% (n = 6) in the AD group, 9.8% (n = 5) in the MCI group, and 3.9% (n = 2) in the SCD group had <20 teeth.

The regression analyses found no apparent associations between the number of teeth and the different outcomes. Even though some point estimates were compatible with an association, they were imprecise. For example, contrasting AD and controls, the crude OR was 1.3 with a 95% CI of 0.4 to 4.1, which remained positively weak after adjustments.

In **paper II**, the number of teeth was used as a binary variable to explore a potential association between tooth loss and changes in the microbial composition, but no associations were found.

In **paper IV**, the exposure was STL (n = 19,927) and contrasted with non-STL (n = 261,659). The median follow-up was 7.9 years. At the end of follow-up, 6.2% (n = 1232) of the STL group and 3.3% (n = 8641) of the non-STL group had developed dementia (all-cause). The incidence density rates for all-cause dementia were 8.3 per 1000 person-years in the STL group and 4.4 per 1000 person-years in the reference group. The median MMSE scores were also lower for those with STL than those without (21 versus 22).

In Figure 4, a Kaplan-Meier plot depicts the relationship between tooth loss and the incidence of all-cause dementia. The two exposure groups differ with regard to dementia incidence. The instantaneous incidence rate was much greater among the STL group compared to the non-STL group.

Using Cox regression, we found an association between STL and all-cause dementia incidence; and the crude HR was 1.89 (95% CI 1.78 to 2.01). After adjusting for age, sex, civil status, disposable income, level of education, and CCI and stratifying for geographic regions, the aHR was 1.16 (95% CI 1.09 to 1.23).

The subgroup analysis showed an association between STL and VaD (aHR 1.75 [95% CI 1.51 to 2.04]). Associations were also found between STL and AD and mixed dementia. By analysing the exposure data using more categories (0, 1-9, 10-19, or ≥20 [reference] teeth), a dose-response association was evident. The following aHRs were demonstrated for 0 teeth (1.27 [95% CI 1.11 to 1.44]), 1-9 teeth (1.18 [1.10 to 1.26]), and 10-19 teeth (1.11 [1.05 to 1.16]). The results were robust after the exclusion of edentulous participants. The introduction of a "wash out" year showed that the results were still robust.

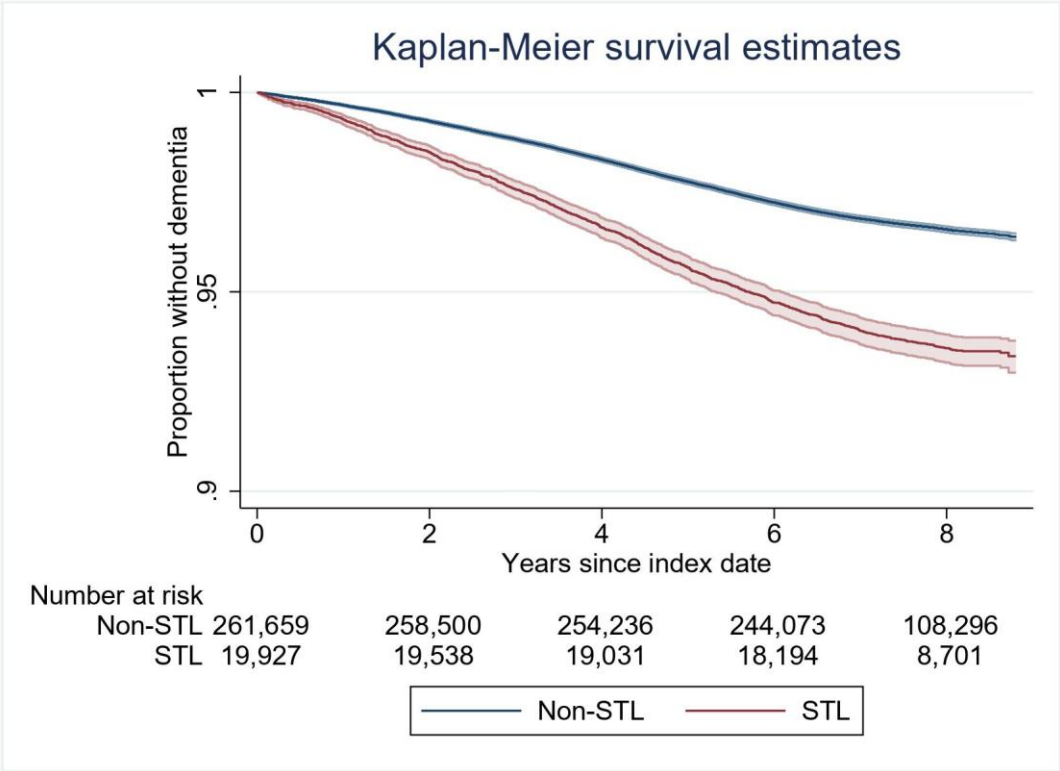

Figure 4. Kaplan-Meier plot of the relationship between severe tooth loss (STL) and dementia. Shaded areas indicate 95% confidence intervals.

Thus, in the case-control study using cross-sectional data and small sample size, no association was found between tooth loss and dementia. Using a longitudinal study design with a large sample size, we found an association between tooth loss and dementia, in which VaD represented the likely driver of the relationship.

6 DISCUSSION

6.1 PERIODONTAL DISEASE, COGNITIVE DYSFUNCTION, AND DEMENTIA

In **paper I**, we hypothesised that poor oral health, especially periodontitis, would be more prevalent among cases with cognitive dysfunction than among cognitively healthy controls. The findings in **paper I** supported the hypothesis and showed that generalised MABL was more prevalent among cases than controls. The association held after adjusting for different covariates assumed to confound the relationship. Similar studies, either case-control studies with mainly cross-sectional data or cross-sectional studies, have shown similar results.[206–208] A Swedish cross-sectional study also used bone loss based on analyses of panoramic radiographs to ascertain exposure and showed an association between low MMSE scores and bone loss.[207] Furthermore, a study in Spain reported data indicating an association between clinical measures of periodontitis and cognitive dysfunction.[206] Another Spanish case-control study with a comparable sample size as our study used a composite variable to define periodontitis by combining CAL and PPD measurements, showing that cases had more periodontal inflammation than controls.[208] In addition, the investigators related the periodontal diagnosis to different biomarkers associated with neurodegeneration and demonstrated associations between periodontitis and IL-6, CRP, and various Aβ peptides in a dose-response manner. Increased CRP levels during midlife have been associated with cognitive decline.[209] Several studies have shown that periodontitis could cause increased serum CRP levels, proving another feasible mechanism linking the diseases.[210]

In **paper I**, three different diagnostic subgroups were investigated; it was apparent that all groups had worse oral health than controls. The AD group had a particularly high prevalence of generalised MABL compared to the other groups. In contrast to what could have been expected, no trend emerged showing worsening oral health on the SCD-MCI-AD spectrum. One can only speculate as to the reason for this. This may be a consequence of sample variability, as the study size is small, or represent actual differences.

Even though **paper I** was based on cross-sectional measurements, radiographic bone loss could be considered a proxy for chronic periodontitis exposure, especially in more severe or advanced states. However, it could be argued that the periodontal tissue destruction occurred after the onset of cognitive dysfunction due to the long latent periods of dementia.[84,211] In **paper I** and Gil-Montoya et al., exposure measurements were performed after the diagnosis of cognitive impairment or dementia, as inherent in the study designs, and thus did not provide any support for the temporal sequence for periodontitis to influence the development of dementia. Studies relying on cross-sectional data to examine chronic exposures and outcomes will be susceptible to reverse causality, resulting in temporality issues. Causal questions could be studied in cross-sectional studies if the exposure is time stable, such as using genes as the exposure.[212] Nevertheless, the discussed studies convey important descriptive information on the oral health state of the participants in the studies, which seems to indicate that poor oral health was more common in these populations, possibly as a consequence of the cognitive impairment. In addition, as will be discussed later in this

chapter, there are biases related to the results that need further discussion for proper interpretation of **paper I**.

In **paper III**, some of the weaknesses in **paper I** could be addressed. For example, we could be certain that the outcome (in the form of a diagnosis of dementia) occurred after the exposure. In **paper III**, the hypothesis was that the incidence of dementia would be higher among those with periodontitis. However, we could not demonstrate any association; periodontitis did not increase the risk of dementia incidence. We had information on most known confounders, except smoking exposure, so we adjusted for possible confounders but it did not affect the results. Smoking exposure can be found in Swedish registers through the Swedish Medical Birth Register, which contains information about the mother's smoking habits during pregnancy. However, it would not have provided decisive information in **papers III** and **IV**. It is likely that comorbidities could serve as a proxy for smoking. The statistical models were adjusted for CCI as a summary index of the burden of comorbidities, but it is likely there is some residual confounding, which can go in both directions.

Population-based studies based on register-data in Taiwan have shown consistent associations between periodontitis and dementia.[121] The studies conducted in Taiwan are similar in design, relying on secondary data sources and design elements. Recently published studies based on the ARIC study in the US have provided inconclusive results.[129,132] An interesting finding in the study by Demmer et al. was that the association was dependent on the definition of periodontitis. When the PPC definition was used, an association was apparent, but no association was found when periodontitis was ascertained based on the well-known CDC/AAP definition. A limitation in **paper III** is that only PPD information was available, which is often regarded as being inferior to CAL or MABL for ascertaining a periodontitis case.[47] To assess whether the findings in **paper III** were sensitive to different exposure levels, we performed a sensitivity analysis with a lower threshold for periodontitis. However, we found no dramatic changes in the estimates. We also performed similar sensitivity analyses for the reference group, but it did not change the nature of the association.

We applied an age restriction of 40 to 80 years. The lower limit was chosen to include participants actually at risk of dementia, even though the dementia incidence is very low among 40 to 50-year-olds. The upper limit reduced the influence of comorbidities and the risk of including individuals with undiagnosed dementia, as it becomes prevalent among those aged >90 years.[213] Some studies do not use age restrictions with an upper limit, which is a possible explanation for the between-study heterogeneity.[214] The follow-up period of 8 years in **paper III**, a result of the establishment of the SKaPa, could be too short for periodontitis to cause dementia. The peak incidence of periodontitis occurs at about 40 years of age.[4] As the dental health registries collects more elapsed time, the follow-up time will increase and baseline exposures could be defined at a much earlier stage, perhaps even before any pathological changes in the brain can be identified.

6.2 SUBGINGIVAL MICROBIOTA IN INDIVIDUALS WITH COGNITIVE DYSFUNCTION

In **paper II**, we found similarities in the subgingival microbial composition across study groups looking at the top 10 most common bacterial genera. The alpha diversity analysis disclosed that the cases had higher richness, typical for periodontitis.[61] When assessing the between-group differential abundance, some notable differences emerged. *Prevotella oulorum*, a Gram-negative anaerobe rod that is only sparsely mentioned in the literature, was more common among cases than controls.[215] In a study investigating experimental gingivitis, *Prevotella oulorum* was associated with gingivitis.[216] *Rothia aeria* was more common in controls. The genera Rothia has been associated with periodontal health.[57] In the subgroup comparisons, two OTUs were more abundant among AD participants than controls: *Slackia exigua* and *Lachnospiraceae* [G-7] bacterium. *Slackia exigua*, a Gram-positive anaerobic rod, has been associated with periodontitis, a fact that was also evident in **paper II** as the OTU was associated with deep periodontal pockets.[217] Another finding was related to *Lachnospiraceae* [G-7] bacterium. There is a sparsity of studies investigating the *Lachnospiraceae* [G-7] bacterium. The Lachnospiraceae family has been described concerning gut health and has also been associated with systemic disease, for instance, depression.[218]

Few studies have explored the composition of the subgingival microbiota in relation to dementia or cognition. A small pilot study (n = 10) suggested alterations in the microbiota when contrasting individuals with normal cognition, individuals who are cognitively impaired without dementia, and those with dementia.[219] A newly published study investigated whether subgingival dysbiosis and AD biomarkers in cerebrospinal fluid (CSF) are associated.[220] The cross-sectional study included 48 participants enrolled from a random sample. Subgingival samples were collected using a curette, and the taxonomic identification was performed by sequencing the 16S rRNA gene. The investigators used a "dysbiotic index" to study the relationship with Aβ42 and found an association between increased levels of subgingival dysbiosis and low Aβ42 levels. They also studied whether subgingival dysbiosis was associated with tau (another CSF biomarker), but no association was found. In the differential abundance analyses, the abundance of certain species was increased among participants with low Aβ42 levels, most notably *Prevotella oris*, *Porphyromonas endodontalis,* and *Prevotella dentocola*, supporting the association between periodontitis and biomarker levels as a proxy for dementia or cognitive dysfunction.

Limited evidence is available on the oral microbiota in relation to dementia. Existing studies differ in study design and methods, hampering comparisons. For example, a study previously mentioned used the V3 region for taxonomic classification[219], whereas in **paper II** and the study by Kamer et al. the V3 and V4 regions were sequenced. The choice of target regions for 16s rRNA sequencing is important and could affect conclusions.[221] In **paper II**, the sampling sites were chosen based on the deepest or most representative periodontal pocket, which favours active periodontitis sites. Thus, the microbial community will be biased towards a periodontitis-associated composition. An uneven periodontitis exposure

distribution across groups would impair inferences, which is a limitation in **paper II**. Other important aspects to consider when comparing different studies concern inclusion and exclusion criteria, which are often strict (e.g., exclusion of antibiotic treatment within the last six months etc.), and the choice of sample technique. The two dominating techniques for the collection of subgingival samples are paper point and curette, which reflect different aspects of the subgingival microbiota. The paper point will mainly collect bacteria that are planktonic or in the outer layer of the dental biofilm, whereas the curette will retrieve a complete sample of the dental biofilm with all layers and, likely, planktonic cells. In **paper II**, curette sampling was used, giving us a representative sample of the entire subgingival dental biofilm and some planktonic cells.

6.3 TOOTH LOSS AND DEMENTIA

Counting teeth is a basic registration in dentistry. It is usually straightforward, though there are situations when registration can be challenging, often because of tooth replacements that obscure the abutment tooth or dental implant. Tooth loss typically represents cumulative dental disease experience, mainly due to dental caries, periodontitis, or trauma, but also congenital conditions.

We found no association between tooth loss and cognitive dysfunction in **paper I**. Cross-sectional studies on tooth loss and dementia or cognitive decline have been summarised and show rather consistent results with positive associations.[222] Tooth loss was not the primary exposure variable in **paper I**; thus, the study was possibly not adequately sized for that specific research question. Another case-control study with a larger sample size, that was discussed earlier, did not find an association between tooth loss and dementia.[206]

In **paper IV**, a register-based cohort study was conducted using STL as the index condition. With a median follow-up of eight years, it was apparent that tooth loss was associated with an increased risk of dementia. This finding held regardless of exposure definitions and adjustments for confounders, and seem to be consistent with prior longitudinal studies.[223] The current knowledge about tooth loss and its link with increased dementia incidence do support the hypothesis that tooth loss is a risk marker for future dementia. Nonetheless, the association is to be regarded as non-causal because there is residual confounding and, in this design, we cannot prove the proper temporal sequence due to a rather short observation period. In a study with up to 37 years of follow-up, fewer teeth were associated with incident dementia, supporting the importance of tooth loss as a risk marker.[224] Other aspects that hinder further inferences are time-related biases and survival bias, which have not been taken into consideration.

Furthermore, in the subgroup analyses, STL was strongly associated with VaD, representing the likely driver of association. Thus, from a mechanistic viewpoint, the association may be mediated through atherosclerosis (or arteriosclerosis). A recent systematic review and meta-analysis showed that tooth loss is also associated with atherosclerotic cardiovascular diseases and mortality.[225]

As previously mentioned, tooth loss represents a cumulative state with many potential causes, and the underlying causal network goes beyond dental caries and periodontitis (downstream factors). For example, smokers have an increased risk of tooth loss, whereas high education has a protective effect, and prior tooth loss is associated with a higher risk of future tooth loss, even after adjusting for periodontitis and dental caries.[226] Tooth loss could also result from economic restraints, as tooth extraction is often a more affordable treatment option than advanced tooth-preserving interventions. The complex causal structure also complicates the interpretation of tooth loss as exposure because it is likely a mediator in a potential causal pathway that combines the effects of several different exposures, some of which may affect dementia risk. In **paper IV**, we could not determine what caused the tooth loss, which also limits further inferences.

6.4 METHODOLOGICAL CONSIDERATIONS

6.4.1 Causal inference and nonexperimental study designs

Aetiological research is distinct from prediction research and descriptive epidemiology.[227,228] The latter do not aim to explore causal effects, as is the case for the former. The demarcation between association and causality is in practice challenging to state, and there is often a hesitation in using the term "causal" in connection with observational research.[229] Historical approaches to causal inference have comprised frameworks with different causal criteria or considerations, the most famous may be Hill´s causal considerations.[230] Modern causal inference concerns methods and theoretical frameworks that work in concert to make inferences on causality. In epidemiology, often used methods and concepts are the sufficient-component cause model, potential-outcome (or counterfactual) model, graphical models using DAGs, and different statistical models (e.g., use of instrumental variables and marginal structural models).[231] Central to causal reasoning is counterfactuals.[232] What would have happened if the exposure did not happen (with everything else the same)? We would have to break the time-space continuum to answer that question, but the difference between the two scenarios represents the causal effect. For the same individual, we will never know.

Central in causal inference is to achieve exchangeability, one of the assumptions for causal inference, which is that the participants are exchangeable across groups (no confounding) or comparable in every aspect except the exposure. Randomisation can set the risks for the outcome to the same level across groups; thus, factors associated with the outcome will be balanced if the randomization is properly implemented. Due to practical and/or ethical reasons, not all research questions can be answered using experiments (i.e., randomised controlled trials [RCTs]). Nonexperimental studies can be used instead, but they are susceptible to several biases that can be difficult to overcome. However, the RCT is also subject to several biases that can have a negative impact on the accuracy (e.g., non-compliance, dropouts etc.).[233] In observational research, causal inference is a demanding endeavour and typically involves many assumptions.[231] A helpful way of designing a nonexperimental study is to use the "target trial approach".[234] The target trial approach makes

us specify how a hypothetical RCT would have been designed and analysed for the current causal question we want to answer.

As epidemiology moves towards studies focusing on causal inference using the potential-outcome framework, it also brings concerns, and a "pluralistic approach" to causality and causal inference has been advocated instead.[235] Thus, the focus should not just be on causal inference using RCTs or studies very close to RCTs. The evidence needs to rest on study diversity and proper synthesis of knowledge from different perspectives and divisions of research.

6.4.2 Systematic error

6.4.2.1 Information bias

Information bias concerns errors in measurements. Information biases result in misclassification, which can arise in the exposure, outcome, and/or covariate status. Furthermore, misclassification can be either differential or nondifferential, and further described as dependent or independent. Careful planning and study design can prevent information (and selection) bias to a large extent. In addition, there are quantitative methods that help deal with bias.[236,237]

Biases that arise in ascertaining exposure could be due to observer bias or recall bias. In **paper I**, oral health was poorer overall among cases compared to controls. As the clinical examinations could not be performed in a masked manner with regards to study group affiliation, the measurements were prone to observer bias because the examiner was aware of the research question, which could lead to differential misclassification of the exposure. Radiological images are a great resource in research. The interpretation of radiographs is also prone to subjectivity and observer bias, but probably less so than clinical examinations because the assessments can be more easily masked to study group affiliation. It is also possible to re-analyse data in a way that is not possible for clinical examinations. Health-related data, socioeconomic status, and data on confounders were collected in **papers I** and **II** using a questionnaire, which could be at risk for recall bias or misunderstanding of questions etc. An alternative could have been to collect covariate data from other sources (e.g., registers and/or patient charts).

An issue in periodontal research is the inconsistency in the definitions of periodontitis cases between studies.[238] In the three thesis papers studying periodontitis (**papers I**, **II**, and **III**), we used different surrogate measures of periodontitis. In **paper I**, we did not formally specify a periodontitis case definition but used a radiological classification of three levels of MABL to assess the prevalence of cumulative periodontitis exposure. In **paper II**, having one or more PPDs ≥ 6 mm served as a proxy for current periodontitis exposure. In **paper III**, DPPD was used as a proxy for periodontitis. Thus, **paper I** represent a cumulative state of periodontitis, whereas **papers II** and **III** were based on current or ongoing periodontal inflammation. It is evident in the literature that study-specific periodontitis case definitions are common practice, some use bone loss,[125,164,165] CAL,[206] PPD, or composite indices.[239] An example of a widely

used classification system is the CDC/AAP classification, a three-level system that involves a combination of PPD and CAL, separately or as a composite index.[131,240] Other examples are the Community Periodontal Index of Treatment Needs and the more recent periodontal profile phenotype (P^3) system.[130] In the new EFP/AAP classification, a periodontal case definition was introduced that mainly defines periodontitis using CAL.[47] All of the different methods and systems for classifying periodontitis are a long-standing problem and impact results depending on which classification system is used.[241,242] The measurements for assessing the presence of periodontitis need to be reliable, valid, and consistent across studies.[243] Misclassification of exposure in **papers III** and **IV** is likely nondifferential because the exposure ascertainment was performed independent of the outcome.

There are several limitations in analysing the characterization of microbiota using next-generation sequencing.[244] In **paper II**, there were risks for bias in the laboratory process. Strict protocols were followed to detect contamination (e.g., collecting samples for replication) and for sample storage, and taxonomic classification methods were used that have been validated.

To identify incident dementia cases in **papers III** and **IV**, we collected outcome information from several sources. However, even though we did not rely on one data source, many with dementia do not seek care and remain undiagnosed and missed in the follow-up.[245] SveDem is based on clinical diagnosis, which is at risk for errors, but few diagnoses changed during follow-up (less than 5%), indicating a valid initial diagnosis.[98]

6.4.2.2 Selection bias

Selection bias involves several biases that produce a difference between the selected study sample and the intended study sample.[246] Potential selection biases that apply in this thesis are discussed in this section.

Participation rates in epidemiological studies have decreased in recent years, which could have a negative impact and lead to nonparticipation bias.[247] Nonparticipation bias arises when participation is associated with the research question under scrutiny. In **paper I**, the participation rate was more than 20 percentage points lower than the cases group. This could introduce bias if the nonparticipants differ from those who accepted the invitation to participate. Certain factors are known to be related to response rates; for example, higher socioeconomic status has been associated with higher participation rates in epidemiological studies.[247] Socioeconomic status is inversely associated with periodontitis, speculatively leading to underestimating periodontitis experience in the control group. In **papers III** and **IV**, the SKaPa provided the exposure information. The SKaPa is based primarily on data from the public dental care service in Sweden (Folktandvården), with population coverage of approximately 40-50%.[179] Thus, a large proportion of the adult Swedish population is treated by private dental care providers, and only a small fraction was included in **papers III** and **IV**.[248] This may have introduced selection bias since individuals attending the public dental care service may differ from those attending private dental care providers, even though

differences generally seem to be small.[249] About 80% of the adult population in Sweden visit a dental care provider regularly.[250]

Matching in case-control studies (as in **paper I**) introduces a selection bias by design. This is because the matching is done on the outcome, which is not the case in cohort studies, in which the matching is done on the exposure and not the outcome. In the case-control study, matching makes the exposure status more similar and will deviate from the source population (the control group exposure distribution should ideally mimic that of the source population). Thus, adjusting for the matching variables is often recommended in case-control studies. It is also important to note the limited advantage of matching in case-control studies for confounder control.[156]

Informative censoring is often a reason for selection bias in cohort studies. Informative censoring can be thought of as differential loss of participants during follow-up.[231] All people are registered in the nationwide registers in Sweden, and the impact of informative censoring is usually low (**papers III** and **IV**). Another source of potential selection bias was the use of restrictions to control confounding, but the extent of bias introduced in restricted samples in cohort studies seems to be small.[251] In addition, selective survival (or collider stratification bias) represent an important bias in dementia research.[252] If we condition on old age, we include participants who survived to old age, which could bias the estimates.

6.4.2.3 Confounding

An important aspect to consider, that often hinders causal interpretations, is confounding. Confounding can be defined using different theoretical concepts.[231] In a DAG, a confounder has been described as a backdoor path between an exposure and outcome.[253] Using the counterfactual model, confounding can be discussed in relation to exchangeability.[254] Confounding can also be described as a mixing of effects, or when a third variable influences the exposure-outcome relationship and gives rise to a noncausal association.[255] Several methods and study design elements can be used to control for confounding. One of the most effective ways to deal with confounding is randomisation, but in nonexperimental studies, one must often rely on a combination of restriction, matching, stratification, and/or regression.

Papers I, **II**, **III**, and **IV** all rely on regression and restriction as the main confounder-control. Covariates that represent confounders, and are included in the statistical model, can be selected in different ways.[256] In the studies included in this thesis, the main method was theoretical reasoning. Therefore, we used prior knowledge with the support of graphical models to construct a network interconnecting the exposure-outcome relationship with assumed confounders that were regarded as causes of the exposures and outcomes and was not a mediator between the exposure and outcome. **Papers I**, **II**, and **III** also use matching, an effective way to control for confounders in cohort studies. In case-control studies, matching leads to selection bias, which must be dealt with during the analysis stage. One problem with using matching (and restriction) is that the sample size gets smaller, affecting

the precision. Another aspect to consider in adjusting for a confounder is time and how the confounder changes over time (i.e., time-varying confounders). Residual confounding is probably evident in all studies, as there are imperfections in the confounder measurements and the existence of unknown or unmeasured confounding. Thus, we are careful in making causal statements.

6.4.3 Random error

If we assume no systematic biases, random error or statistical variation is left to deal with, usually described as "chance", to get the sample estimand as close as possible to the true population value. Random error has been defined as random variations in the measurements that originate from various sources, which often are difficult to identify.[160] Sampling variability is one potential source. Sampling variability arises because of random variation in the sampling process (i.e., each sample includes different individuals, so there will be some differences between samples).

The classical way of determining the influence of random error is to report P-values that quantify whether the observed data are compatible with a null hypothesis. According to the ASA statement, a P-value (probability value) is defined as "the probability under a specified statistical model that a statistical summary of the data (e.g., the sample mean difference between two compared groups) would be equal to or more extreme than its observed value."[257] The P-value is based on a test hypothesis (usually a null hypothesis) and assumes that the assumptions of the statistical model are met and no bias exists in the study. Hence, assumptions are rarely met in clinical research. The P-value is continuous but has been subject to arbitrary dichotomization using alpha levels that determine a cut-off that shows whether the result is "significant" (and that one should reject the null hypothesis). Misuse and misinterpretation of the P-value have received much criticism.[158,257,258]

An alternative to P-values is estimation, which determines CIs along with the point estimate. Even though CIs have been subjected to misconceptions, they give the reader more information than a P-value. A 95% CI can be interpreted as follows: If we collect data from many, many different samples, the true value that we want to estimate will be within the two confidence limits 95% of the time.[259] The CI confers information regarding precision, and the width is closely related to sample size. Thus, a large sample size reduces random error and results in a narrow CI. Using Swedish nationwide registers as data sources in **papers III** and **IV** enabled substantial sample sizes, positively impacting the precision. It is important to emphasize that the accuracy of the point estimate and CI is also dependent on systematic biases (for instance, measurement error), not solely on random error. The sample size in studies with primary data collection is often limited by time and resources. Problems with precision can be dealt with during the planning stage using sample size calculations.[260]

A limitation in **paper I** concern the rather small sample size. In addition to the imprecise estimates due to the sample size, there is also a risk of sparse data bias. Sparse data bias results from few observations of exposures, outcomes, and/or covariates, which leads to bias

in the regression estimates.[261] It is not only a problem in small studies but also in studies with large sample sizes. For example, few observations of generalised MABL were found in the study groups in **paper I**. The change in estimates and increasing uncertainty (broader CIs) that comes with adjustments in the SCD group could represent biased estimates due to sparse data bias.

6.4.4 Generalizability/transportability

It is essential to have valid results for a broader population, a target population, beyond the study population.[156,262] Generalizability describes whether the results in the sample represent the same estimates as in the source population. Transportability describes whether the sample estimates are valid for another population that is not part of the population from which the sample was collected. The two most influential aspects on generalizability are the presence of bias and differential distribution of effect modifiers.

Papers I and **II** were based on a highly selected case population and a control group randomly selected from Huddinge municipality. Furthermore, the sample size was small and prone to sample variability. There is probably a lack of generalizability in **papers I** and **II**, which is further supported by the low participation rates. **Papers III** and **IV** are population-based nationwide studies that include people attending dental care in Sweden, with a high frequency of visits, indicating good generalizability if the target population is people living in Sweden.[263] However, the results in **papers III** and **IV** may not be transportable to other age groups. Existing bias in all papers could negatively impact the generalizability.

7 CONCLUSIONS

The findings of this thesis add important knowledge on the relationships between periodontal disease, tooth loss, cognitive dysfunction, and dementia. The work also shows the complexity of oral-systemic disease interactions, especially as both the exposure and outcome often comprise chronic diseases with long (or unknown) induction and latency periods. Other issues are exposure definitions, which are seldom consistent across studies, and confounding. The main strengths of this thesis were the use of different methods, types of data collection, study designs, and populations to investigate the exposure-outcome relationship between periodontitis, tooth loss, cognitive dysfunction, and dementia. The papers in this thesis also demonstrate the advantages and disadvantages of different study designs in the context of non-experimental aetiological research.

The findings were dependent on the type of study design. In the case-control study based on cross-sectional data, an association between periodontitis (defined as MABL) and cognitive dysfunction was evident, but no association for teeth counts and the outcome. In contrast, the two longitudinal studies showed an inverse relationship. Thus, there was no association between periodontitis and dementia, but instead between tooth loss and dementia.

In conclusion:

- Signs of periodontitis were more common among participants with AD, MCI, or SCD compared to controls. Using MABL as the primary exposure of interest, generalised MABL was associated with cognitive dysfunction, particularly AD.

- The use of a longitudinal study design with a large sample size showed that exposure to deep periodontal pockets (a proxy for periodontitis) did not lead to an increased incidence of dementia during an 8-year follow-up. Neither statistical adjustments nor sensitivity analyses changed the nature of the association.

- The subgingival microbial composition did not dramatically differ across study groups, though we did find some differences that warrant further investigation. One important finding was that bacteria associated with periodontal disease were more abundant in participants with cognitive dysfunction, which also validated the clinical findings in **paper I**. Future studies on the subgingival microbiota should try to recruit participants with a more similar distribution of periodontal disease, as the varying distribution complicated inferences in our study.

- In a cross-sectional setting, no association could be demonstrated between tooth loss and cognitive dysfunction.

- In a longitudinal setting, severe tooth loss was associated with an increased risk of dementia. This finding was demonstrated in a population-based, nationwide cohort

study with up to nine years of follow-up. The results were robust after adjusting for potential confounders and in sensitivity analyses. The relationship may be confounded or prone to unknown biases, but we suggest severe tooth loss is a risk marker for future dementia development.

8 POINTS OF PERSPECTIVE

In the absence of a cure for dementia and a probable increase in dementia prevalence from a global perspective, it is of the utmost importance to identify new modifiable aetiological risk factors for dementia. Even with a cure, prevention is usually preferred, especially if the preventive strategy comes with a low cost, easy administration, and adds other beneficial health effects to the individual. Multidomain preventive trials have been published, showing promising results.[264] Dementia is a multifactorial disease in which different components work together to cause disease, which also is the case for periodontitis. If a causal relationship can be demonstrated between dental disease and dementia, it is likely that the magnitude of the effect is rather small (**paper IV**). A recent commentary supports this notion.[265] The authors exemplified the likely weak causal effect of periodontitis on systemic diseases using the sufficient-component cause model or causal pie model.[156] In the causal pie model, several component causes constitute a causal mechanism (termed sufficient cause). The pie represents the sufficient cause with its components. The component causes can differ between individuals (e.g., different component causes for the same outcome). It is possible that poor oral health could be a weak cause of dementia. Even if a hypothetical effect size would be small, it could be important from a public health perspective if the intervention is cost-effective and added to an already existing preventive strategy.

Sweden and the Nordic countries are great for register-based research. The SKaPa and DHR are great dental health registries but are relatively new, and there is still much that could be improved. Many essential variables are not available, which complicates ascertaining exposures, outcomes, and covariates. For example, regarding periodontitis, data on loss of attachment (CAL or bone loss) would be a great addition. An extension of the SKaPa is in process, a register for dental implants. Another useful extension of the SKaPa could be the SveDem model for data collection, which could be implemented for specialist clinics in periodontology. The data could include detailed information on diagnosis, classification, and radiographs that could be analysed for research purposes. Likely wishful thinking, but a more specialised quality register for periodontitis has the potential to increase the management of periodontitis patients and answer other related research questions. Another aspect, which was already mentioned, is that the oral health registers are rather new. As more time elapses, the longer the observation periods will become and, with these, more valid inferences.

The published literature on oral-systemic disease interactions generally uses traditional epidemiological approaches to study relationships, usually based on single-centre data with small or moderate sample sizes in cross-sectional settings. Unfortunately, this approach will seldom add valuable scientific information in the context of causation and sometimes even represent research waste.[266,267] To quote Altman in 1994: “We need less research, better research, and research done for the right reasons.”[268]

Refined registers (especially for dental care), further interdisciplinary collaborations, thoughtful study design, and new methods in large-scale studies will allow valid causal conclusions and generalizable results. The field of causal inference has undergone impressive

advancements since the 1980s, with the introduction of formal causal models, DAGs, and new statistical tools and methods.[231] As experimental studies can seldom be conducted due to practical and ethical restraints, observational studies need to confer more valid inferences on causality in the context of oral-systemic disease interactions. I believe that interdisciplinary collaboration among subject matter specialists, methodologists, and biostatisticians needs to be mandatory in future research. In addition, a greater focus on multi-centre studies, or the combination of nationwide register data and researcher-generated data, is of paramount importance.

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