Dental status as a window to general health

H.C.M. (Marie-Chris) Donders

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CHAPTER 1

1

General introduction
and outline of the thesis

GENERAL INTRODUCTION

The history of dentistry is almost as ancient as the history of civilization with the earliest evidence dating from 7000 BC. From the Middle Ages until the 19th century, dentistry was not yet a profession in itself, but an occupation of barbers and the surgeons. They were actually one entity, named barbers surgeon, and were responsible for a range of services relating to care of the body. A barber surgeon performed surgical procedures including amputations, bloodletting and tooth extractions, as well as barbering roles like hair cutting and shaving. Tooth extraction, similar to bloodletting, was used as a therapeutic as well as a prophylactic process, supposed to remove toxins from the body and to balance the "humors". The link between dentistry and general medicine seems historical. However, since the establishment of the first dental college (Baltimore College of Dental Surgery) in 1840, dentistry became a separate entity from medicine.[1] This separation has been maintained by divided education, divergent practices, payment models, and health care policies. Nevertheless, the connection between dental health and general health is in the middle of a revival and its importance is now realized worldwide with major impact on public health.

Remarkable epidemiological and pathological associations between dental status and general health have been reported. Most research focused on the link between periodontitis and cardiovascular disease but also with many other systemic diseases including diabetes mellitus, rheumatoid arthritis, certain cancers, respiratory diseases, cognitive disorders and premature birth.[2] The first study in modern times that found evidence for the association between dental pathology and cardiovascular disease was by Mattila et al. in 1989.[3] This initial study caused a wave of commotion and was leading to the provocative quote "Floss or die".[4] Since then a multitude of studies on this topic have been published, though the fundamental explanations for the associations remained under debate.[5] Most precedent literature tried to find causality between dental pathology and systemic diseases, mainly based on derivative parameters. This thesis provides new insights into this link elaborated in two essential general health conditions: cardiovascular diseases (as the leading cause of global mortality) and COVID-19 (as a recent example of a worldwide pandemic). In this introductory chapter, "dental status" and these two crucial general health conditions are further explicated.

Dental status

Oral diseases are one of the most prevalent diseases globally.[6] The key clinical dental conditions that are considered to be public health priorities include dental caries and periodontitis. Eventually, tooth loss is the ultimate event representing dental pathologies. Tooth loss at a younger age is generally due to caries, and in older ages, it is the final stage of periodontitis. In 2010, 2.3% of the global population, was edentulous (no natural teeth). Prevalence of severe tooth loss (≤9 remaining teeth) reduced between 1990 and 2010, declining from 4.4% to 2.4%. However, this prevalence increases gradually with age, showing a steep increase around the seventh decade of life, associated with a peak in the incidence of severe tooth loss at the age of 65. This older age pattern of tooth loss has not changed during

the past two decades, notwithstanding the gradual decreases in prevalence and incidence within the same period for the whole population.[7]

Dental caries is the primary cause of oral pain and the prevalence of ever having had caries in adults is high, reaching more than 90% of the population.[8] Dental caries is the localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates. Physical and biological risk factors for dental caries include inadequate salivary flow and composition, high numbers of cariogenic bacteria, insufficient fluoride exposure, gingival recession and genetic factors. It is a chronic disease that progresses slowly in most people and is initially reversible. In dental caries management, the focus has been around prevention, but tooth restauration and tooth extraction is still widely used.[9]

Untreated caries leads to bacterial invasion of the pulp and root canal. This condition may progress with necrotic root canals and resorption of apical periodontal ligament and surrounding alveolar bone. These peri-apical lesions contain bacteria which can be translocated throughout the body and lodge in various organs.[10]

Periodontitis is the sixth most common human disease, affecting 30-50% and approximately 10% of the global adult population in its most severe form.[11] The global age-standardized prevalence and incidence have remained stable since 1990.[12] Periodontitis is a chronic multifactorial inflammatory disease of the supportive tissues of the teeth. It starts with localized inflammation of the gingiva that is initiated by bacteria in the dental plaque. This gingival inflammation (gingivitis) can be present for years and is considered as a normal and protective host response. Nevertheless, in highly susceptible individuals and with progression age, the host response may show a break in the tolerance to the dental microbiome along and just below the gingival margin. Eventually, due to lack of proper immune fitness, gingivitis may derail in a destructive form of gingival inflammation. This subsequent state is periodontitis, with periods of exacerbation showing progressive loss of alveolar bone and tooth attachment. The inflammation actually creates a favorable ecosystem for pathobionts and a dysbiotic biofilm develops. Although pathogenic bacteria in the dysbiotic biofilm are necessary for periodontitis to take place, a susceptible host is also needed. Consequently, several risk factors for periodontitis have been established, including smoking, diabetes mellitus, socio-economic position, psychosocial factors and genetic predispositions.[13]–[15]

Timely diagnosis of periodontitis is extremely important, since loss of the periodontal tissues is largely irreversible. However, the most prevalent form of periodontitis is painless and it is common to have reached advanced degrees of severity before it is diagnosed. After proper diagnosis and classification, periodontal treatment consists of non-surgical root debridement followed by a surgical treatment phase to further reduce residual deep periodontal pockets. In extreme cases or cases in young individuals, some clinicians opt for adjunctive therapy with systemic antibiotics. Patient education in proper oral hygiene and counselling on control of risk factors for periodontitis, together with a periodontal maintenance programme of 3-4 times a year is important for secondary prevention.[13], [16]

During periodontitis, pathogens in the dysbiotic biofilm trigger immune responses involving both innate immunity as well as adaptive immunity resulting in the production and release of pro-inflammatory molecules. In this regard, it is interesting to notice that inflammation here plays a dual role: Inflammatory response is a physiological reaction aimed at protecting the organism against bacterial infections. However, when inflammation becomes deregulated and chronic, it may lead to an irreversible destruction of the periodontal tissues and becomes the frontline allowing local inflammation to disturb systemic health.[17]

Periodontitis has gained relevance since it has been shown that it can develop into a systemic condition. Unresolved periodontal hyperinflammation may cause, coincide or exacerbate other health issues associated to elevated morbidity and mobility and mortality.[17] Most research in this field focused on the association between periodontitis and cardiovascular diseases, but recent analyses of trial registers showed that even fifty-seven systemic conditions are hypothesized to be linked with periodontitis.[18]

Cardiovascular disease

Cardiovascular disease (CVD) is a cluster of disorders of the heart and blood vessels, including coronary heart disease, cerebrovascular disease and peripheral arterial disease. CVD is the leading cause of global mortality and a major contributor to disability.[19] Acute events of CVD such as myocardial infarction (heart attack), cerebrovascular accident (stroke), and sudden death are mainly caused by an obstruction of the blood vessels. The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels, called atherosclerosis. The term atherosclerosis derives from the Greek word for 'gruel' or 'porridge', reflecting the appearance of the lipid material found in the core of the typical atherosclerotic plaque. This underlying pathology, atherosclerosis, is a progressive chronic inflammatory process of the arteries, characterized by a dysfunctional interplay between the immune system and lipids. The observation that inflammatory cells are interspersed in the atheroma was made in the late 1800s, but the contribution of immune cells to all stages of atherosclerosis began to be valued only in the last few decades.[20] Numerous studies have clarified the molecular mechanisms of inflammation in atherosclerosis, and it is widely accepted that both innate and adaptive immune responses play key roles in the initiation and progression of atherosclerosis, leading to clinical manifestations of CVD.[21]

Immune cells, as well as smooth muscle cells, platelets and endothelial cells, drive plaque inflammation through a complex crosstalk of inflammatory mediators. These mediators are activated by risk factor–induced triggers, which are present in the circulation and in the vessel wall, such as shear stress, oxidized lipoproteins and oxidative stress. Without relief from risk factors, the activation of inflammatory processes persists, resulting in a chronic non-resolving inflammation. Inflammation is associated with severity of disease, and complex lesions, which are prone to rupture and cause acute events, are characterized by extensive inflammation.[22]

The relevance of several major risk factors for CVD is now well established, including, but not limited to, smoking, obesity, hypertension, hypercholesteremia, diabetes mellitus and genetics.[23] Because of the multifactorial nature of CVD, its treatment should target all known treatable risk factors. Ideally, primary prevention starts by adopting a healthy lifestyle, reducing exposure to the avoidable major risk factors. Nevertheless, risk factor modification to prevent or even reverse the progression of the atherosclerotic process can provide benefit at any stage of atherosclerotic disease, additionally in the context of secondary prevention.[24]

Consistent epidemiological evidence additionally indicates periodontitis as a risk factor for CVD. The explanation of this association between periodontitis and CVD generally fall into two categories: (a) microbial mechanisms, which through vascular invasion may locally affect the development of the atheroma lesions; and (b) inflammatory and immunologic mechanisms that directly influence the pathobiology of the atheroma lesions.[25] Whether or not treatment of periodontitis is valuable for primary or secondary prevention of cardiovascular disease, have not yet been fully established.[26]

CVD and periodontitis are both complex inflammatory diseases considerably influenced by similar multilevel interactions between metabolic and immune systems. The susceptibility of a host and its associated aberrant immune response is considerably the fundament of this link. In this scope, much more inflammatory diseases and their related complications could be linked. The recent detected, and still ongoing COVID-19 pandemic has significantly increased our focus and perceptions of inflammatory conditions , immune responses and its consequences.[17]

Coronavirus disease 2019 (COVID-19)

At the end of 2019, the novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first detected in China. The later designated coronavirus disease 2019 (COVID-19) rapidly developed in a worldwide pandemic presenting an important and urgent threat to global health.[27] Countries around the world reported 4.2 million deaths from COVID-19 from the beginning of pandemic until the end of July 2021, but the actual number of deaths is probably higher.[28] The most common serious complication of COVID-19 infection, Acute Respiratory Distress Syndrome (ARDS), is characterized by bilateral chest radiographical opacities with severe hypoxemia due to non-cardiogenic pulmonary oedema.[29] Furthermore, COVID-19 does not only affect the respiratory tract, but it also affects other organs with multi organ failure as endpoint. Admission to the Intensive Care Unit (ICU) for mechanical ventilation is predominantly necessary; not primary to cure, but to allow time for the body to recover. Corticosteroids (dexamethasone) can help reduce the length of mechanical ventilation and save lives of patients with severe and critical illness. Nevertheless, approximately one-third of the patients admitted to the ICU with a severe form of COVID-19 eventually die.[30]

The COVID-19 pandemic forced the world to accelerate vaccine and drug development and evaluation at an unparalleled pace. At present, the COVID-19 treatment arsenal is largely represented by antiviral agents (often administered in early stages of disease) and immunotherapeutic agents that modulate the host immune response (often administered in more advanced stages of disease).[31] Moreover, many different public organizations and private companies have worked together to make COVID-19 vaccines available. While the rapidly developed COVID-19 vaccines have provided strong protection against serious illness, hospitalization and death, around 40% of the worldwide population is still unvaccinated until February 2022.[32], [33]

Indication of risk factors for a severe course of COVID-19, such as hospital admission, ICU admission and death, became crucial. Therefore, there was an urgent need for a pragmatic risk stratification tool that allows the early identification of the COVID-19 patients who are likely to be at highest risk of ICU admission and death.[34] Age is one of the main risk factors for morbidity and mortality due to infection with SARS-CoV-2.[35] Additionally, male sex, underlying medical conditions (cardiovascular-, metabolic-, lung- and renal-disease) and obesity are associated with COVID-19 related complications and unfavorable outcomes.[36] It has been hypothesized that poor oral health is associated with the severity of the clinical progression of COVID-19.[37], [38] Consequently, tooth loss, as ultimate sequala of poor oral health and dental pathology, could possibly serve as an easily accessible biomarker for the early identification of COVID-19 patients at risk for a severe disease progression, ICU admittance and even death from COVID-19. Accordingly, also for COVID-19 the dental status could serve as window to general health.

OUTLINE OF THE THESIS

Above it has been outlined that the interface between dental status and general health is fascinating and relevant. Most precedent literature in this field tried to find causality between dental pathology and systemic diseases, mainly based on derivative parameters. This thesis provides new insights into this link elaborated in two essential general health conditions: cardiovascular diseases (as the leading cause of global mortality) and COVID-19 (as a recent example of a worldwide pandemic). Herewith, a more widespread and general statement on this exciting and above all important topic is made.

Therefore, the aim of this thesis is threefold. First, the association between dental status and cardiovascular disease is further investigated using more adequate parameters **(Part I)**. Secondly, the possible link between dental status and severity of COVID-19 is explored **(Part II)**. In the last part, the link between dental status and general health is discussed based on Part I & II **(Part III)**.

Part I – Dental status as a window to cardiovascular disease

Chapter 2 is a review of the literature on the association between periodontitis and atherosclerosis and provides the state of knowledge in this field. The retrospective study presented in **Chapter 3** investigates the association between Coronary Artery Calcium (CAC) scores defined on CT scans and dental pathology seen on dental panoramic radiographs. In **Chapter 4,** a prospective clinical study determines if there is a correlation between the inflammatory burden of periodontitis (quantified by the Periodontal Inflamed Surface Area [PISA] score) and the presence and extent of coronary calcification (investigated by the CAC score). The secondary aims were to study other cardiovascular parameters and CVD risk predictors in relation to periodontitis and dental status. **Chapter 5** describes the effect of periodontal treatment on endothelial function and other cardiovascular parameters after one-year follow-up of the same patients investigated in chapter 4.

Part II – Dental status as a window to COVID-19

In **Chapter 6** the association between alveolar bone loss, tooth loss and severity of COVID-19 is explored in a retrospective study. **Chapter 7** describes the development and external validation of a prediction model for critical outcomes of COVID-19, based on dental status in addition to the established risk factors such as demographic characteristics and other medical condition.

Part III – Dental status as a window to general health

In **Chapter 8** the results of the various chapters of this thesis are discussed and clinical implications and future perspectives are given. **Chapter 9** and **Chapter 10** presents the summary of this thesis in respectively English and Dutch.

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PART I

Dental status as a window to cardiovascular disease

CHAPTER 2

Review Article
The association between periodontitis and atherosclerosis
The current state of knowledge

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J. de Lange

This chapter is based on the publication in:
Journal of Cranio- Maxillary Diseases
2012

Abstract

Atherosclerosis is a chronic inflammatory condition and infectious diseases are believed to contribute to its pathophysiology. Periodontitis is a chronic infectious disease of the supporting tissues of the teeth, and the epidemiological association with atherosclerosis is now beyond doubt. However causal mechanisms are still lacking; research suggests that bacteria from the periodontal lesions may enter atherosclerotic plaques. Alternatively, elevated CRP and a prothrombotic state in periodontitis contribute to exacerbation of atherosclerosis. Finally, the link may also be explained by polymorphisms in the ANRIL gene, which has been associated with both atherosclerosis and periodontitis.

Previous studies used surrogate biomarkers to investigate the association between atherosclerosis and periodontitis, and to evaluate the effects of periodontal intervention. Unfortunately, more definitive cardiovascular parameters are still lacking, because of methodological difficulties in study design and ethical considerations.

Introduction

Cardiovascular disease is the leading cause of death and morbidity in the Western world. Atherosclerosis, a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries, constitutes the single most important contributor to this growing burden of cardiovascular disease.[1] Over the past two decades, inflammation has emerged as an integrative factor for atherosclerosis. Inflammation can operate in all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis.[2]

Periodontitis is a chronic multi-causal inflammatory disease of the supportive tissues of the teeth with progressive loss of attachment and alveolar bone.[3] Periodontitis is the most common oral disease and affects 10–30% of the general population, depending on age.[4] The first study that found positive epidemiological evidence for the association between periodontitis and atherosclerosis was in 1989 by Mattila et al.[5] Thereafter, in more recent years, remarkable pathological and epidemiological associations between these two diseases have been presented.[6]

This review summarizes the epidemiological and clinical evidence for the association between periodontitis and atherosclerosis and assesses the current state of knowledge regarding the suggested biological mechanisms to explain this association. A very important topic, with regard to the high incidence of both diseases, their economic costs to society and the potential impact on public health, if risk modification or therapeutic opportunities could be identified.[7]

Methods

A literature search was carried out using MEDLINE with language restriction to English. We used free-text search terms and the Boolean operators “OR” and “AND”: [“periodontal disease” OR “periodontitis”] AND [“atherosclerosis” OR “atherosclerotic” OR “coronary heart disease” OR “cardiovascular disease”]. Additionally, we searched manually in the reference list of the articles, obtained by the electronic search, for other relevant articles.

Biological mechanisms for the association between periodontitis and coronary heart disease

Several pathophysiological pathways have been suggested to explain the association between periodontitis and atherosclerosis. These pathways involve both direct and indirect mechanisms.

Indirect mechanism: Increased level of systemic inflammation

Periodontitis is associated with increased levels of C-reactive protein (CRP), fibrinogen tumor necrosis factor-α, IL-1, Il-6, IL-8 and other acute phase reactants.[8] These inflammatory reactants promote systemic inflammation and are associated to atherosclerosis. In case of systemic inflammation, endothelial cells stimulated by these inflammatory reactants increase their expression of various leukocyte adhesion molecules. Once adherent to the activated endothelial layer, the monocyte moves between the endothelial cells to penetrate into the innermost layer of the arterial wall and initiates an atherosclerotic lesion. Once resident in the arterial intima, monocytes acquire the morphological characteristics of macrophages, undergoing a series of changes that lead ultimately to foam cell formation. These foam cells are lipid-laden macrophages and characterize the early atherosclerotic lesion. Macrophages within atherosclerotic plaques also secrete a number of growth factors and cytokines involved in lesion progression and complication.[2]

Indirect mechanism: Increased platelet activation

Periodontitis is associated with increased p-selectin and platelet activation.[9] P-selectin functions as a cell adhesion molecule on the surfaces of arterial endothelial cells and activates platelets, in response to inflammation. Periodontopathogens are able to directly cause activation of endothelial cells and platelets.[10] Since platelet activation contributes to a pro-coagulant state and constitutes a risk for atherothrombosis, platelet activation in periodontitis may partly explain the epidemiological association between periodontitis and atherosclerosis.[9]

Indirect mechanism: Molecular mimicry

Molecular mimicry has been raised as a possible mechanism linking periodontitis with atherosclerosis. Molecular mimicry is thought to occur when sequence similarities between foreign and self-proteins produce cross-activation of auto-reactive T- or B-lymphocytes that can lead to an autoimmune reaction and tissue damage. In this hypothesis, the induction and progression of atherosclerosis might be explained by the immune response to so-called bacterial heat-shock proteins (HSPs). All cells express HSPs upon exposure to various forms of stress including inflammation. Concerning atherosclerosis, expression of host protective HSP on endothelial cells may be induced by a variety of factors including bacterial lipopolysaccharide, cytokines and mechanical stress.[11] The immune system may not be able to differentiate between self-HSP and periodontopathic bacterial HSP. Therefore, molecular

mimicry suggests that antibodies directed by the host to periodontopathic bacterial HSP may result in an autoimmune response to HSPs expressed on endothelial cells, resulting in endothelial dysfunction and development of atherosclerosis.[12]

Direct mechanism: Invasion of periodontal pathogens into atherosclerotic plaques

Bacteremia that originates from the mouth is a common event that occurs multiple times a day while chewing and tooth brushing, especially in patients suffering gingivitis and periodontitis.[13] Periodontal pathogens (i.e. Porphyromonas gingivalis, Aggregatibacter Actinomycetemcomitans, Prevotella intermedia, Treponema denticola and Eikenella corrodens) enter the circulation via the gingival sulcus. These periodontal pathogens adhere to and invade in vascular endothelial cells. Infection of these endothelial cells by the periodontal pathogens (in particular Porphyromonas gingivalis) induces a procoagulant response that might contribute to formation of an atherosclerotic plaque.[14] Moreover, periodontal pathogens have been found in atherosclerotic plaques.[15]

Potential genetic mechanism

The recent identification of a shared genetic locus, ANRIL, for periodontitis and atherosclerosis is a factor of unknown influence, but could be even more important than the above proposed biological mechanisms.[16] The function of ANRIL and its role in periodontitis and atherosclerosis is still lacking.

Epidemiological evidence for the association between periodontitis and atherosclerosis

Several epidemiological studies have confirmed the association between periodontitis and atherosclerosis. The first study that found positive epidemiological evidence for this association was in 1989 by Mattila *et al*.[5] Bahekar *et al*.[17] recently summarized the subsequent studies in a systematic review revealing five prospective cohort studies (follow-up >6 years), five case-control studies and five cross-sectional studies. Meta-analysis of the five prospective cohort studies (86092 patients) indicated that individuals with periodontitis had a 1.14 times higher risk of developing coronary heart disease (CHD) than the controls (relative risk 1.14, 95% CI 1.074–1.213, $P < 0.001$). The case–control studies (1423 patients) showed an even greater risk of developing CHD (OR 2.22, 95% CI 1.59–3.117, $P < 0.001$). The prevalence of CHD in the cross-sectional studies (17724 patients) was significantly greater among individuals with periodontitis than in those without periodontitis (OR 1.59, 95% CI 1.329–1.907, $P < 0.001$). The individual studies could well be adjusted for confounding factors, because of the extensive documented impact of many prevalent risk factors, shared by periodontitis and CHD. These shared risk factors include increasing age, male sex, race/ethnicity, education and socio-economic status, stress, smoking, alcohol abuse, diabetes mellitus and overweight.[18,19]

Clinical evidence in the literature for the association between periodontitis and atherosclerosis

Observational studies using surrogate endpoints

Periodontitis can correctly be diagnosed and controlled by intra-oral examination (gingival bleeding, pocket-depth, loss of attachment and microbiological sampling or analysis) and dental X-ray (loss of alveolar bone). However, to strictly diagnose atherosclerosis, there is a need of invasive techniques such as angiography of the coronary arteries. Today, several surrogate biomarkers and imaging tools for atherosclerosis are in clinical and experimental use. Since inflammation has emerged as an integrative factor for atherosclerosis, epidemiological studies have found increased vascular risk in association with increased levels of inflammatory biomarkers such as cytokines (IL-6, TNF-α), cell adhesion molecules (P selectin) and acute-phase reactants (CRP, fibrinogen), which are elevated in periodontitis patients.[8] Growing evidence indicates that elevated circulating inflammatory markers, in particular CRP, are predictors for an unfavorable course, independent of the severity of the CVD or inflammatory burden.[2] Paraskevas *et al*.[20] showed in a meta-analysis of 10 cross-sectional studies that the weighted mean difference of CRP between periodontitis patients and controls was 1.56 mg/l ($p < 0.00001$).

Besides, there are a number of other non-invasive surrogate subclinical markers of cardiovascular disease, focused on the endothelial function and arterial stiffness, including measurement of the carotid arteries, echocardiography, ankle-brachial index, flow-mediated dilation (FMD) in the brachial artery and pulse waveform analysis.[21] Söder *et al*.[22] found significantly higher mean values of the common carotid artery intima-media thickness (IMT) and calculated intima-media area (cIMA) in patients with periodontitis than in controls, both at the right ($P < 0.01$ and $P < 0.001$, respectively) and left side ($P < 0.001$ for both variables). Carotid IMT increase is associated with a raised risk of CHD.[23] Endothelial dysfunction precedes clinical manifestation of atherosclerosis. Flow-mediated dilation (FMD) of the brachial artery assesses the endothelial function and is decreased in subjects with atherosclerosis. Amar *et al*.[24] displayed that subjects with advanced periodontitis had lower FMD compared with control patients (7.8 +/- 4.6% versus 11.7 +/- 5.3%, $P = 0.005$).

Observational studies using hard endpoints

Only a few studies considered periodontitis with hard endpoints of atherosclerosis such as first occurrence of death from CHD, hospitalization due to CHD, or revascularization procedures. Hujoel *et al*.[18] studied a total of 8032 dentate adults aged 25 to 74 years with no reported history of cardiovascular disease. After adjustment for known cardiovascular risk factors, periodontitis was associated with a non-significant increased risk for CHD event (hazard ratio, 1.14; 95% confidence interval, 0.96–1.36). Jansson *et al*.[25] found in a prospective study with

a sample of 1393 subjects, after a follow-up of at least 20 years that the extent of bone loss due to periodontitis was a risk indicator of death due to CHD. For individuals younger than 45 years of age, the age-adjusted incidence odds ratio of death due to CHD was 2.7 (p = 0.04) if subjects with mean marginal bone loss of >10% were compared with subjects with mean marginal bone loss < or =10 %.

Clinical trials concentrated on atherosclerotic risk reduction after periodontal treatment

Whether or not periodontal treatment reduces the risk for atherosclerosis or complications of atherosclerosis have not yet been established. The majority of the intervention trails, aimed to study this purpose, has examined the effect of periodontal treatment on markers of systemic inflammation or surrogate biomarkers of atherosclerosis. A recent meta-analysis on C-reactive protein in relation to periodontitis has indicated that periodontal treatment resulted in a weighted mean reduction in serum CRP of 0.5 mg/l (95% CI 0.08–0.93, p = 0.02). This reduction leads to clinical relevant improvements in systemic inflammation.[20] Tonetti *et al*.[26] sought to assess the effect on intensive periodontal treatment on endothelial function measured by FMD of the brachial artery. Twenty-four hours after treatment, FMD was significantly lower in the intensive-treatment group than in the control-treatment group However, FMD was greater in the intensive-treatment group than in the control-treatment group 60 days after therapy (absolute difference 0.9%; 95% CI, 0.1 to 1.7; P = 0.02) and 180 days after therapy (difference, 2.0%; 95% CI, 1.2 to 2.8; P < 0.001). The degree of improvement was associated with improvement in measures of periodontal disease. A recent pilot study reported the effect of periodontal treatment on changes in carotid IMT. A group of healthy subjects suffering from mild to moderate periodontitis was treated with root debridement. Six and twelve months after treatment, IMT was significantly decreased at different locations in the carotid artery.[27]

Conclusion

The association between periodontitis and atherosclerosis is of great public health importance because of the high prevalence of both diseases and the potential impact on public health if risk modification or therapeutic opportunities could be identified. Extended review of the literature focused on this subject suggests that periodontitis is associated with atherosclerosis, independent of known confounders. Previous studies use surrogate biomarkers in order to investigate the association between periodontitis and atherosclerosis. Unfortunately, more definitive cardiovascular parameters and endpoints are still lacking.

Several biological pathways have been suggested to explain this association; however, causal mechanisms are still not demonstrated. Polymorphisms in the ANRIL gene, which has been associated with both atherosclerosis and periodontitis, might be an important factor.

Several studies have evaluated the effects of periodontal treatment on endothelial function, in particular improvement of the cardiovascular condition through an increase of flow-mediated dilation (FMD) after periodontitis intervention has been observed. However, these studies utilize the brachial artery, which is a surrogate for the condition of coronary arteries. Besides, only subjects with severe periodontitis have been included in these clinical trials of periodontal therapy and endothelial function improvement. Therefore, there is still no available evidence that periodontal treatment improves endothelial function in subjects affected by the more prevalent forms, i.e. slight or moderate periodontitis. Studies exploring the effects of periodontal therapy in atherosclerotic patients with periodontitis are needed because treatment studies with periodontitis patients without atherosclerosis have shown benefits for cardiovascular system. However, due to ethical reasons, it is not possible to do intervention RCTs, treating periodontitis in an experimental group and using an untreated control group, with longitudinal follow up to score cardiovascular events. In conclusion, further studies are required to identify clinical relevant aspects of the association between periodontitis and atherosclerosis. Well-designed interventional trials should demonstrate the short-term and longitudinal effect of periodontal treatment on the emergence and progress of atherosclerosis and cardiovascular events.

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CHAPTER 3

Elevated coronary artery calcium scores are associated with tooth loss

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Abstract

Aim

This study explores the association between Coronary Artery Calcium (CAC) scores and dental pathology such as missing teeth, the (peri-apical) health status and restoration grade of the teeth, and the grade of alveolar bone loss seen on a dental panoramic radiograph (Orthopantomograph – OPG).

Materials and Methods

In this retrospective cross-sectional study, data was collected from three hospitals spread in the Netherlands. Patients were included when a CAC score and an OPG were available, both recorded within a maximum period of 365 days from 2009-2017. The CAC score was measured on a CT scan, using the Agatston method. To assess dental pathology, the number of missing teeth, the number of dental implants, alveolar bone loss, caries, endodontic treatments, peri-apical radiolucencies, bone loss at implants, impacted teeth and dental cysts, were determined on the OPG. All observers were calibrated. The electronic health records provided information about: gender, age, smoking, Diabetes Mellitus, hypercholesterolemia, hypertension and Body Mass Index (BMI).

Results

212 patients were included. We found a statistically significant association between the number of missing teeth and the CAC score. When modeling age, sex, and other well-known risk factors for cardiovascular disease, the significant correlation was no longer present after multivariate correction. Furthermore, the results showed a trend for more teeth with peri-apical lesions and a higher percentage of mean alveolar bone loss in the group with the highest CAC scores.

Conclusion

This study showed that being edentulous or missing teeth is correlated to higher CAC scores however failed to be an independent predictor of atherosclerotic cardiovascular diseases. The number of (missing) teeth is an easily accessible marker and could be used as a marker for atherosclerotic cardiovascular disease (ACVD) risk by almost any healthcare worker. The current study needs to be considered as an explorative pilot study and could contribute to the design of further (prospective) studies on the relationship between dental pathology and coronary artery calcification by adding clinical information and extra cardiovascular biomarkers.

Introduction

Atherosclerotic cardiovascular diseases (ACVD) are one of the leading causes of death and morbidity in the Western world.[1] The underlying pathology, atherosclerosis, is a progressive disease characterized by the accumulation of lipids and fibrous elements in the arteries. Over the past two decades, inflammation has emerged as an integrative factor for coronary atherosclerosis. Inflammation can be evident in all stages of this disease, from initiation through progression and, ultimately, the thrombotic complications of coronary atherosclerosis.[2]

Remarkable epidemiological and pathological associations between oral health and cardiovascular diseases have been reported. The first study that found evidence for the association between dental pathology and coronary heart disease was in 1989 by Mattila et. Al..[3] Since then a multitude of cross-sectional and longitudinal studies have implicated periodontitis as a risk for ACVD in addition to the well-known risk factors including smoking, hypercholesterolemia and diabetes.[4]

Tooth loss is the ultimate event representing dental pathologies. Various articles have found that missing teeth are predictors of incident CVD, for example as was reported by Liljestrand et al..[5] More recently an interesting meta-analysis on tooth loss and risk of ACVD and stroke was published.[6] Dental caries is a lifelong disease and traditionally considered as an important cause of tooth loss. Dental caries has a multifactorial etiology; consumption of dietary carbohydrates, composition of the oral flora and poor oral hygiene are the most important etiological factors. Frencken et. al determined in their systematic review a global age-standardized prevalence of untreated dentine carious lesions in the permanent dentition of 35% There were no significant differences between sexes and disease prevalence reached its peak at age 25, with a second peak later in life at around 70 years of age.[7]

Another dental pathologic condition that can lead to tooth loss is apical periodontitis. This is a chronic inflammation around the apex of a tooth, in most cases caused by bacterial invasion of the pulp and root canal, most often as a result of untreated dental caries. This condition is frequently asymptomatic and may progress with the resorption of apical periodontal ligament and surrounding alveolar bone; some cases flare up, however most peri-apical lesions are discovered on routine dental x-ray's during dental checkups. These peri-apical lesions contain bacteria which can be translocated throughout the body and lodge in various organs and also in atherosclerotic lesions.[8][9] Nevertheless there are only a few studies that have suggested an association between chronic apical periodontitis and cardiovascular disease.[10]

Periodontitis on the other hand, is a chronic multi-causal inflammatory disease of the supportive tissues of the teeth with progressive loss of attachment and alveolar bone, finally leading to tooth loss.[11] It is the most common oral disease, affecting 30-50% of the adults and approximately

10% of the population in its most severe form.[12] Quite some research has been performed to identify pathophysiological mechanisms to explain the association between periodontitis and coronary heart disease.[13] Recently an update on the association and plausible mechanisms how periodontitis can be a risk factor for ACVD has been published.[14]

Since inflammation has emerged as an integrative factor for cardiovascular disease, many studies used biochemical inflammatory biomarkers such as cytokines (IL-6, TNF-α), cell adhesion molecules (P-selectin) and acute-phase reactants (CRP, fibrinogen) as surrogate parameters for cardiovascular risks.[15] However, in addition to inflammatory biomarkers, there are a number of clinical non-invasive surrogate markers of cardiovascular disease. These are related to the endothelial function and arterial stiffness, including measurement of the carotid arteries, echocardiography, ankle-brachial index, flow-mediated dilation (FMD) in the brachial artery and pulse waved velocity analysis.[16] These surrogate cardiovascular biomarkers have been widely used to explore the association between dental pathology and cardiovascular diseases.[17]–[19]

Nowadays, Coronary Artery Calcium (CAC) scoring has emerged as a widely available, consistent and reproducible means of assessing risk for major cardiovascular outcomes, especially useful in asymptomatic people for planning primary prevention interventions.[20] Coronary artery calcium provides superior discrimination and risk reclassification of cardiovascular disease in intermediate-risk individuals, compared with ankle-brachial index, high-sensitivity CRP and family history.[21] CAC scoring has sporadically been used to investigate the association between periodontitis and cardiovascular diseases. [22] [23] The current retrospective cross-sectional pilot study explored the association of Coronary Artery Calcium (CAC) scores with radiographic parameters of dental pathology, including missing teeth, periodontal disease, dental caries and peri-apical disease.

Materials and methods

For this retrospective cross-sectional study, data were collected from three hospitals on different locations in the Netherlands (Academic Medical Centre, Amsterdam; Isala Hospital, Zwolle; Ziekenhuis Gelderse Vallei, Ede). Patients were included when the hospital data provided a CAC score and a dental panoramic radiograph (Orthopantomograph – OPG), both obtained within a maximum period of 365 days between them, from 2009-2017. All data were anonymized before accessed. This study was approved by the Medical Ethical Committee (15.06107) of the Isala Hospital, Zwolle and accepted by the other participating hospitals. The Medical Ethical Committee waived the requirement for informed consent.

Patient characteristics

The electronic health records provided information about: sex, age, smoking, diabetes mellitus, hypercholesterolemia, hypertension and body mass index (BMI). When diabetes mellitus, hypercholesterolemia or hypertension were not mentioned in a patient file, but the corresponding medication was available (e.g. metformin and/or insulin, statins and antihypertensive drugs), the patient was scored positively for that disorder. BMI was calculated with the noted height and weight on the day of the CT-scan for the CAC score.

Coronary Artery Calcification

Most of the included patients received a CAC CT-scan because of presentation with symptoms suspected for myocardial ischemia. The CAC scan of the heart was rapidly acquired, prospectively electrocardiogram-triggered and without contrast. The CAC score was quantified using the Agatston method where the area of calcified atherosclerosis (defined as an area of at least 1 mm^2 with a CT density >130 Hounsfield units [HU]) is multiplied by a density weighting factor and summed for the entire coronary artery tree using a 2.5 to 3.0 mm slice thickness CT dataset.[24]

Dental pathology

To asses dental pathology, the following markers were evaluated on the OPG: number of missing teeth, number of dental implants, alveolar bone loss, caries, endodontic treatments, peri-apical radiolucencies, bone loss around dental implants (as a sign for peri-implantitis), impacted teeth and dental cysts. A total of 5 observers were involved in assessing the OPGs and calibration was conducted as follows. Observers A and B (2 dentists, trained by a periodontist) scored the number of present teeth, dental implants and the alveolar bone loss. Observers A and B were calibrated by comparing individual scorings of 10 random OPGs. The results were mutually evaluated, to roughly calibrate the two examiners. Subsequently, 10 new OPGs were scored individually and the agreement was statistically determined. This intra-class correlation coefficient was 0.87. After four weeks the intra-examiner reliability was determined. The same

10 OPGs were scored again and compared with the scores from 4 weeks earlier. The intra-examiner reliability was 0.76 for observer A and 0.73 for observer B. According to Fleiss, scores between 0.4 and 0.75 represent fair to good reliability and scores higher than 0.75 represent excellent reliability.[25]

Observers C, D and E (2 oral and maxillofacial surgeons and an endodontist) scored caries, restorations, endodontic treatments, peri-apical radiolucencies, bone loss at implants, impacted teeth and dental cysts. Peri-apical radiolucencies (osteolytic lesions) and dental caries were recorded as present or absent without consideration of size.[26] When in doubt, "present" was assigned. To calibrate the observers, twenty OPGs (randomly selected from the database) were scored and the agreement for each variable was statistically determined by calculating a Cohen's Kappa value. The intra-examiner reliability was 0.98 for caries, 0.90 for restorations, 1.0 for endodontic treatments, 0.89 for peri-apical radiolucencies, 0.74 for bone loss at implants, 1.0 for impacted teeth and 0.73 for dental cysts.

Number of missing teeth

The number of present teeth was measured by counting all teeth visible on the OPG, including third molars and radices relictae. Pontics of fixed partial dentures and prosthetic dentures were not counted as teeth. The number of missing teeth was calculated by subtracting the number of present teeth from the expected total of 32 teeth. Dental implants were counted individually.

Alveolar bone loss

To score the loss of alveolar bone for each tooth, the cemento-enamel junction (CEJ), the alveolar crest and the apex of the root had to be visible. Using a modified Schei ruler, the loss of alveolar bone was measured in tenths of percentages of the root length. In this study the distance representing the biological width was determined at 2 mm on the Schei ruler, based on the used magnification factor of the printed OPGs, instead of the 1 mm in the conventional Schei ruler, used for intra oral radiographs.[27][28] Both the mesial and the distal sites were measured. The highest score of each tooth was used for analysis. To determine the alveolar bone loss of a tooth, the transparent Schei ruler was placed on a printed OPG with the marking of the biological width at the CEJ landmark, perpendicular to the longitudinal axis of the tooth and was moved until the last radius covered the apex landmark. The amount of alveolar bone loss was then determined by identifying the position of the alveolar crest relative to the markings of the ruler. For teeth decayed or restored beyond the CEJ, the cervical margin of the decay or restoration was used as the CEJ landmark. For dental implants, the most apical outline of the crown and the apical end of the implant were used as respectively the CEJ and apex landmarks.

Statistics

Descriptive statistics (mean ± standard deviations [SD] or numbers [%] of subjects) were used to present patient characteristics and clinical findings. The Shapiro-Wilk test was used for the calculation of the normality of distribution of CAC scores. The patients were grouped in tertiles based on the CAC scores. The mean numbers of the dental pathologies scored on the OPG were calculated per group and possible differences between groups were tested by ANOVA. A backward stepwise linear regression model with variables with $p<0.01$ to stay, was applied to explore any contributing dental factor that appeared to have an uni-variate significance with CAC scores in relation to traditional cardiovascular disease risk factors (age, sex, BMI, diabetes, hypertension, hypercholesterolemia, smoking). For the latter analysis, CAC scores were log transformed to better approach normality of data distribution. Analyses were performed using IBM SPSS Statistics 26 software (SPSS Inc., Chicago, IL, USA). P-values <0.05 were considered significant.

Results

We retrieved 212 patients with an available CAC score and an OPG both recorded within a maximum period of 365 days between them. In 121 (57.1%) patients, the CAC score was assessed before the OPG, in 89 (42.0%) patients the OPG was first available and in 2 (0.9%) patients the CAC score and OPG were taken at the same day. The mean intermediate period between these two radiographic investigations was 170 days (SD 127 days).

The background characteristics of these patients are presented in Table 1. The population of this study consisted of 54% (n=114) male patients. The mean age was 57.8 years (SD 12.2 years). The mean BMI was 28 kg/m^2 (SD 4.9 kg/m^2). Fifteen percent (n=32) of the patients were diabetic, 40% (n=85) of the patients suffered from hypercholesterolemia, and 60% (n=128) of the patients were treated for hypertension. The smoking status and smoking history for all patients was divided into three categories: 41% (n=86) of the patients had never smoked, 41% (n=86) of the patients were past-smokers and 18% (n=39) of the patients were current smokers. There was no information available about the period of time the past smoker patients had been smokers (pack-years).

We observed that 70 (33%) patients had a zero CAC score while the remainder (n= 142) had CAC scores ranging from 1, up to 20000. This prompted us to stratify all individuals into tertiles (Table 1): group 1 containing the zero CAC scores, group 2 (n=70, 33%) had CAC scores in the range of 1-125, and group 3 (n=72, 34%) had CAC scores ranging from ≥126 to 6141, but also included one outlier subject with a notable CAC score of 20000.

Table 1. Patient characteristics

		Total n= 212	**CAC Tertile 1 n=70**	**CAC Tertile 2 n=70**	**CAC Tertile 3 n=72**
Age (years)		57.8 ± 12.2	50 ± 11	59 ± 10	65 ± 10
Male sex		114 (53.8)	32 (28.1)	32 (28.1)	50 (43.9)
Body Mass Index (kg/m^2)		28.0 ± 4.9	28 ± 5	29 ± 5	27 ± 5
Diabetes mellitus		32 (15.1)	9 (28.1)	10 (31.3)	13 (40.6)
Hypertension		128 (60.4)	34 (26.6)	47 (36.7)	47 (36.7)
Hypercholesterolemia		85 (40.1)	16 (18.8)	34 (40.0)	35 (41.2)
Smoking[a]	Current	39 (18.4)	17 (43.6)	8 (20.5)	14 (35.9)
	Ever	86 (40.6)	21 (24.4)	34 (39.5)	31 (36.0)
	Never	86 (40.6)	32 (37.2)	28 (32.6)	26 (30.2)

Values represent number of subjects (%) or mean ± standard deviation
a: For 1 patient the smoking status was unknown.

The dental findings and the dental pathology in this study population are arranged per CAC-tertile group in table 2. The study population consisted of 43 (20.3 %) edentulous patients and 169 (79.6%) dentate patients. First, we observed a significant higher percentage of edentulous patients in the higher CAC tertile (p=0.009); there were 22 patients edentulous in the latter group, while only 9 and 12 patients in CAC tertile 1 and tertile 2 respectively. The edentulous patients in the highest CAC tertile had significant less implants (for implant retained dentures) than the patients in the lower CAC tertiles (p=0.006); 46% of the edentulous patients in the highest CAC tertile had implants versus respectively 89% and 92% of the edentulous patients in CAC tertile 1 and 2.

169 (79.1%) patients in the study population were dentate. 152 (89.9%) of these dentate patients had only natural teeth and 17 (10.1%) of these patients had a combination of natural teeth and dental implants. The number of missing teeth per CAC tertile was significant (p=0.03); the mean number of missing teeth was 7.6 (SD 6.6) in the lowest CAC tertile and 11.0 (SD 7.6) in the highest CAC tertile. Additionally, the number of teeth with untreated caries was significantly higher in the tertile with the highest CAC scores (p=0.05). Furthermore, the results showed a trend for more teeth with peri-apical lesions and a higher percentage of mean alveolar bone loss in the CAC tertile group with the highest CAC scores, with a p-value of respectively 0.07 and 0.06 All other dental findings were not correlated to the CAC scores and are listed in table 2.

Table 2. Dental conditions

	All subjects n=212	CAC Tertile 1 n=70	CAC Tertile 2 n=70	CAC Tertile 3 n=72	p-value
Edentulous	**43 (20.3)**	**9 (12.9)**	**12 (17.1)**	**22 (30.6)**	**0.009** *$^{\nabla}$
With implants	29 (67.4)	8 (88.9)	11 (91.7)	10 (45.5)	0.006$^{\nabla}$
With implants with bone loss	7 (16.3)	3 (33.3)	2 (16.7)	2 (9.1)	0.109
Dentate	**169 (79.6)**	**61 (87.1)**	**58 (82.9)**	**50 (69.4)**	**0.009** *$^{\nabla}$
Missing teeth	9.4 ± 7.1	7.6 ± 6.6	10.0 ± 6.8	11 ± 7.6	0.033$^{\nabla}$
Dental implants	0.3 ± 1.0	0.2 ± 0.6	0.4 ± 1.5	0.3 ± 0.8	0.397
Implants with bone loss	0.1 ± 0.6	0.1 ± 0.3	0.2 ± 0.9	0.12 ± 0.4	0.440
Restored teeth	12.5 ± 5.5	11.9 ± 5.0	13.1 ± 5.5	12.7 ± 6.2	0.535
Endodontically treated teeth	1.9 ± 2.2	1.7 ± 2.0	1.8 ± 2.4	2.1 ± 2.2	0.640
Teeth with peri-apical lesions	2.9 ± 2.4	2.9 ± 2.6	2.4 ± 2.1	3.5 ± 2.4	0.070
Teeth with caries	3.2 ± 2.7	3.1 ± 3.3	2.7 ± 1.9	4.0 ± 2.7	0.050
Radices relictae	0.5 ± 1.2	0.7 ± 1.5	0.2 ± 0.6	0.6 ± 1.2	0.136
Impacted teeth	0.3 ± 0.7	0.4 ± 0.9	0.3 ± 0.6	0.3 ± 0.6	0.363
Cysts	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.4	0.1 ± 0.3	0.725
Mean alveolar bone loss (%)	21.5 ± 10.7	20.2 ± 11.2	20.1 ± 9.5	24.4 ± 11.1	0.064

Values represent number of subjects (%) or mean ± standard deviation. Group differences were tested with one-way ANOVA. Tertile 1: CAC score = 0, Tertile 2: CAC score 1-125, Tertile 3: CAC score >125. *From the same Chi-square analysis. ∇ Statistical significant, P-value <0.05

Table 3 displays the results of modeling the CAC variation in the study population by a backwards-linear regression. Potential confounders initially included were age, sex, BMI, diabetes, hypertension, hypercholesterolemia and smoking. Tooth loss was the only dental pathology used in this model. Age, BMI and the missing teeth were continuous parameters and all other were categorical parameters. Higher age, male sex and hypercholesterolemia accounted for most of the variance in CAC values. Tooth loss had a standardized Beta correlation coefficient with the CAC scores of 0.11 (versus 0.49 for age, 0.23 for male sex and 0.17 for hypercholesterolemia) and showed a trend to be associated but this failed to reach statistical significance (p=0.079).

Table 3. Final backward linear regression model to explore variations in CAC values among 212 subjects.

	B	p-value
Age	0.49	0.000
Male sex	0.23	0.000
Hypercholesterolemia	0.17	0.004
Missing teeth	0.11	0.079

B = Standardized Beta coefficient
Potential confounders initially included in model; Age, sex, age, BMI, diabetes, hypertension, hypercholesterolemia, smoking. Higher age, male sex and hypercholesterolemia accounted for most of the variance in CAC values

Discussion

This retrospective, cross-sectional pilot study is the first that explored the association and possible correlation between CAC scores and the common dental pathologies. The most obvious and definitive dental pathological event is tooth loss. We observed a statistically significant association between the number of teeth lost and the CAC score. However, when adjusted for age, sex and hypocholesteremia, this correlation was no longer significant (p=0.079). Furthermore, we found univariate trends in dentate patients for an association between higher CAC scores and teeth with peri-apical lesions and untreated caries.

Tooth loss is the ultimate state of dental pathology. Most tooth loss before middle age is caused by dental caries. Dental caries is a disease with a multifactorial etiology; consumption of dietary carbohydrates is one of the most important etiological factors. Carbohydrate intake is also associated with increased risk for cardiovascular diseases and they can therefore indirectly effect each other.[29] Furthermore, tooth loss is the "end point" of periodontal disease. This prolonged state of chronic inflammation with increased levels of C-Reactive Protein (CRP) is a proven risk factor for cardiovascular diseases.[14] Besides, smokers are much more likely to develop periodontitis than non-smokers and smoking has a strong negative effect in response to periodontal treatment.[11] Smoking has therefore a well-known common effect on cardiovascular diseases and tooth loss. Above all, tooth loss might provide harmful health benefits and has been considered to impact quality of life.[6][30]

In the current study we defined the number of teeth by counting the teeth on dental panoramic radiographs (Orthopantomographs – OPGs). The number of present teeth, and correspondingly the tooth loss, is an easily accessible marker and can be determined by anyone; the general practitioner, the dentist or even the patient itself. We assumed that loss of teeth was a result of dental pathology with dental caries and periodontal disease as leading causes. This should be carefully interpreted since in some cases perhaps a tooth may have been lost due to non-pathological causes such as orthodontic treatment, dental trauma and agenesis. However, the incidence of those events is low. The OPGs were also used to determine the number of dental implants, alveolar bone loss, caries, endodontic treatments, peri-apical radiolucencies, bone loss at implants (as a sign for peri-implantitis), impacted teeth and dental cysts. Regarding the alveolar bone loss, intra-oral radiographs are considered the standard for dental radiographic diagnostics. Nevertheless, studies have shown that OPGs and intra-oral radiographs are in great agreement.[31] For the illustration of the actual peri-apical health, a peri-apical radiograph shows a better diagnostic accuracy than an OPG.[32] Similarly, small peri-apical lesions may be better visible on intra-oral radiographs. While the OPG has a high specificity, the sensitivity is low for the detection of apical periodontitis in treated and untreated teeth, especially in the incisor area.[33]

Since only radiographical and no clinical information was obtained to determine the oral health status, no assumptions could be made on the activity of the dental pathology. The observed pathology can be in an active, in a chronic or in a remission state. For example, alveolar bone loss does not necessarily accompany an active periodontitis process. Also, the peri-apical lesions could only be scored on the presence and not on activity. Peri-apical lesions can be active, inactive or a result of a healing process, i.e. a scar from previous flare-ups. However, an inactive process or a "scar" might still have caused an inflammatory process in another part of the body.[34] Previous studies in which they found a relation between (peri-apical) periodontal disease and cardiovascular diseases used clinical information.[9][13]

The maximum time allowed between the OPG and CT scan was one year. However, the average time between these two radiographic assessments was 170 days. We are aware that there is the possibility that all "scored" parameters, both for CAC scores and dental pathology, could have changed in the course of the time difference between them. We assume the pathological processes, calcium deposition as well as progression of dental pathology, are both rather slow processes and changes within 1 year will not be large. For this pilot study we deemed the maximum of 1 year acceptable.

The CAC score is used as a strong and proven biomarker for atherosclerotic cardiovascular diseases. The presence and extent of CAC can predict the presence of coronary artery stenosis, but in general it is a better marker of the extent of coronary atherosclerosis than the severity of the stenosis. However, the absence of CAC (CAC = 0) has been shown to be the strongest "negative risk factor" as compared to normal or negative values of multiple other novel risk markers for future CVD events, including carotid intima-medial thickness (CIMT), absence of carotid plaque, family history, ankle brachial index, B-type natriuretic peptide (BNP), albuminuria, family history, and hsCRP. This "power of zero" provides the strongest degree of individual "de-risking" available as compared to traditional and other novel biomarkers.[35] CAC scoring is especially useful in asymptomatic patients, but CAC also has prognostic value in symptomatic patients. However, in symptomatic patients, a CAC score of 0 does not carry the same high negative predictive value as it does in asymptomatic patients.[36][37] In this study, the vast majority of the included patients were symptomatic.

Conclusion

This study provides suggestive evidence that Coronary Artery Calcium is associated with the ultimate “hard” endpoint of dental pathology, i.e. tooth loss. It should be considered as a pilot study and further studies need to confirm the current findings. Nevertheless, the current findings add to the wealth of research showing the relationship between oral pathology and atherosclerotic cardiovascular diseases, in which tooth loss can be considered as an easy accessible possible marker for cardiovascular and overall health status. Health workers, especially general practitioners, dentists and cardiologists must be aware that tooth loss is sign of poor oral health and that patients with extensive tooth loss may have an increased risk for cardiovascular disease.

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CHAPTER 4

The association between periodontitis and cardiovascular risks in asymptomatic healthy patients.

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Abstract

Background

Periodontitis is a chronic multifactorial inflammatory disease of the supportive tissues of the teeth. Pathophysiological evidence suggests a possible common inflammatory background between periodontitis and cardiovascular diseases (CVD). Pathological and epidemiological associations between these two diseases have been presented, but are still debated. This study aimed to investigate the association between the inflammatory burden of periodontitis and the presence and extent of coronary calcification. Secondary aims were to study other cardiovascular parameters and cardiovascular risk predictors in relation to periodontitis and dental health.

Methods

Healthy periodontitis or non-periodontitis patients 45-70 years of age were included in a prospective cross-sectional study. Full-mouth examinations were performed by a periodontist to determine their Periodontal Inflamed Surface Area (PISA) score and other dental parameters. To assess the cardiovascular conditions, Coronary Artery Calcium (CAC) scores, endothelial function assessments by the EndoPAT ™, and several physical and biochemical examinations were performed.

Results

Seventy-one patients were included. Elevated CAC scores and endothelial dysfunction were not significantly related to PISA or dental health. PISA was significantly related to the Framingham and Reynolds CVD risk predictors, but were no longer significant after correction for confounders. The same applied to the significant relations between tooth loss, dental plaque and bleeding scores and the CVD risk predictors.

Conclusions

Periodontitis is associated with increased CVD risk, but is not an independent risk factor. This link is still important to make to bridge the gap between dentistry and general medicine and to identify patients at risk for CVD in an earlier stage.

Introduction

Cardiovascular disease (CVD) is one of the leading causes of death and morbidity in the Western world.[1] The underlying pathology, atherosclerosis, is a progressive chronic inflammatory process. The observation that the atherosclerotic fibrofatty lesions are supplied with inflammatory cells was made in the late 1800s, but the contribution of immune cells to all stages of atherosclerosis began to be valued only in the last few decades.[2][3] Numerous studies have clarified the molecular mechanisms of inflammation in atherosclerosis, and it is widely accepted that both innate and adaptive immune responses play key roles in the initiation and progression of atherosclerosis, leading to clinical manifestations of CVD.[4]

Periodontitis is a chronic multifactorial inflammatory disease of the supportive tissues of the teeth, with progressive destruction of alveolar bone and tooth attachment ending in tooth loss.[5] As a result of inflammation, the tissues surrounding the tooth are infiltrated by neutrophils, macrophages and activated lymphocytes, releasing cytokines and subsequently acute phase reactants (CRP, fibrinogen).[6] Periodontitis is the most common oral disease, affecting 30-50% of adults and approximately 10% of the population in its most severe form.[7]

Pathophysiological evidence points to a possible common inflammatory background between periodontitis and atherosclerosis.[8] The first study that found positive epidemiological evidence for the association between periodontitis and atherosclerosis was in 1989 by Mattila *et al*..[9] In more recent years, remarkable pathological and epidemiological associations between these two diseases have been presented, though without any final conclusions.[10]–[13]

Since inflammation has emerged as an integrative factor for atherosclerosis, several inflammatory biomarkers, in particular high- sensitivity C-reactive protein (hsCRP), are used as surrogate biomarkers to investigate the association between periodontitis and CVD.[14] However, definitive randomized evidence for the role of hsCRP as a causative biomarker in atherosclerosis is lacking.[15] In addition to biochemical biomarkers, there are a number of non-invasive surrogate subclinical markers of cardiovascular disease, focused on the endothelial cell dysfunction and arterial stiffness, which are used to explore the association between periodontitis and CVD.[16]

We designed a study focused on achieving a more definitive quantification of the association between periodontitis and coronary atherosclerosis by investigating the Coronary Artery Calcium (CAC) score. CAC scoring is a highly specific feature of coronary atherosclerosis, and has emerged as a widely available, consistent and reproducible means of assessing risk for major cardiovascular outcomes.[17] Compared with other surrogate biomarkers, the CAC score provides superior discrimination and risk reclassification of cardiovascular disease in individuals at intermediate risk.[18]

The primary aim of this cross-sectional study was to determine if there is an association between the inflammatory burden of periodontitis (quantified by the Periodontal Inflamed Surface Area [PISA] score) and the presence and extent of coronary calcification (investigated by the CAC score).[19] Secondary aims were to study other cardiovascular parameters and CVD risk predictors in relation to periodontitis and dental health.

Materials and Methods

Study design

This prospective cross-sectional study was approved by the Medical Ethics Committee, Isala Academy, Zwolle, the Netherlands (NL43083.075.13) and has been registered in the ISRCTN trial registry with study ID ISRCTN55656827. All participants provided written consent for participation. This study was done in accordance with the Declaration of Helsinki guidelines for human research, 1964, and amended in 2013 (64th World Medical Association General Assembly, Fortaleza, Brazil). Data were collected, interpreted and analyzed by the authors.

Participants

We included patients, between 45 and 69 years of age, without known systemic diseases and with at least 10 teeth, who visited the Practice for Periodontology Zwolle (PPZ). Patients with diagnosed, untreated periodontitis and patients without (a history of) periodontitis were included.

Measures of dental health

All patients underwent a full-mouth periodontal examination performed by two trained periodontists at the Practice for Periodontology Zwolle (PPZ). These periodontists were calibrated by comparing individual measurements of ten random patients. The statistically determined intraclass correlation coefficient of 0,81 represented an excellent reliability according to Fleiss.[20] Periodontitis was initially diagnosed and staged according to the consensus report of the World Workshop on the classification of periodontal and peri-implant diseases and conditions.[21] The Periodontal Inflamed Surface Area (PISA) score was applied. This scoring tool calculated the amount of inflamed periodontal tissue in square millimeters and quantified the total infectious burden resulting from periodontitis.[19] The PISA score was calculated after extensive periodontal examination, including periodontal probing pocket depth (PD), plaque score and bleeding on probing (BOP). All measurements were performed on all teeth, on six sites per tooth using a manual periodontal standard probe.

Measures of general health

All patients filled out questionnaires to gather data on their medical history, perceived health, parental history, lifestyle, socio-economic status and oral hygiene.

At least two weeks after the periodontal examination, patients were examined by a trained nurse at the Department of Cardiology of the Isala hospital, Zwolle. Physical examinations were performed, and blood pressure (BP), heart rate (HR), body mass index (BMI), waist-to-hip ratio (WHR), and electrocardiogram measurements (ECG) were obtained. Venous blood was collected to determine levels of high sensitive C-reactive Protein (hsCRP[mg/L]), total

cholesterol (mmol/L), HDL-cholesterol (mmol/L), LDL-cholesterol (mmol/L), triglycerides (mmol/L), estimated Glomerular Filtration Rate (eGFR ml/min/1,73m^2) and glycosylated hemoglobin (HbA1c [%]).

Measures of cardiovascular conditions

The presence and extent of coronary artery calcification were investigated by an ultrafast CT scan (LightSpeed VCT XT; GE Healthcare). The CT scan of the heart was rapidly acquired, prospectively electrocardiogram-triggered and without contrast. The CAC score was quantified using the Agatston method, in which the area of calcified atherosclerosis (defined as an area of at least 1 mm^2 with a CT density >130 Hounsfield units [HU]) is multiplied by a density weighting factor and summed for the entire coronary artery tree using a 2.5 to 3.0 mm slice thickness CT dataset.[22]

As secondary outcome we performed an endothelial function assessment by the EndoPAT ™ (Itmar Medical, Israel), based on noninvasive Peripheral Arterial Tone (PAT) signal technology measuring endothelium-mediated changes in vascular tone using bio-sensors placed on the fingertips.[23] The final result of the EndoPAT ™ is the Reactive Hyperemia Index (RHI), which is a ratio of the post-to-pre occlusion PAT amplitude of the tested arm, divided by the post- to pre- occlusion ratio of the control arm. A RHI score of 1.67 and below correlates to endothelial dysfunction.[24], [25]

Cardiovascular risk prediction

The commonly used Framingham risk score (including age, gender, total cholesterol, high-density lipoprotein cholesterol, smoking status, and systolic blood pressure) and Reynolds risk score (including age, current smoking, parental history of a cardiovascular event, <age 60 years, blood pressure, hs-CRP and total and HDL cholesterol) were calculated to predict the risk of a patient having a cardiovascular event in the next 10 years.[26]

The Systemic Coronary Risk Evaluation (SCORE) algorithm, recommended by the European Society of Cardiology (ESC) for CVD risk stratification in asymptomatic individuals, also estimates the individual 10-year risk of death from CVD. SCORE is based on sex, smoking, systolic blood pressure, total cholesterol (mmol/L) and HDL cholesterol (mmol/L).[27] In this study, SCORE was calculated using the online calculating tool HeartScore (https://www.heartscore.org)

The MESA (Multi-Ethnic Study of Atherosclerosis), a prospective community-based cohort study of 6,814 participants age 45 to 84 years, who were free of clinical heart disease at baseline and followed for 10 years, created an algorithm for 10-year CVD risk estimation. An accurate estimate of 10-year CVD risk was obtained using the coronary artery calcium score (Agatston units) and traditional risk factors: age, sex, race/ethnicity, diabetes (yes/no), current

smoker (yes/no), total and HDL cholesterol, use of lipid lowering medication (yes/no), systolic blood pressure (mmHg), use of anti-hypertensive medication (yes/no) and any family history of heart attack in a first-degree relative (yes/no).[28]

The MESA risk score was calculated using the online calculator. (https://www.mesa-nhlbi.org)

Statistics

Descriptive statistics (mean ± standard deviations (SD), Median [IQR] or numbers (%) of subjects) were used to present patient characteristics, behavior and dental and cardiovascular findings. Group differences were tested by one-way analysis of variance (ANOVA), independent T-Tests for quantitative variables or Chi-square analysis for categorical variables. Univariate binary logistic regression analyses (for the dichotomized dependent variable) and univariate linear regression analyses (for the continuous dependent variables) were performed to assess the association between each independent variable and the dependent variables. Multivariate linear regression analysis was performed afterwards for the independent variables that were significant in the univariate analyses. In multivariate regression analysis, the independent variables were adjusted by the most relevant confounders (gender, age, BMI, waist-to-hip ratio, education, alcohol and smoking). If an independent variable did not receive a significant p-value in the univariate analysis, the subsequent multivariate analysis was not presented. The significance level was set at a p-value of 0.05. All analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA)

Results

A total of 41 patients with diagnosed, untreated periodontitis and 30 patients without periodontitis were recruited. The patient characteristics of these 71 patients are presented in table 1. The study population consisted of 43.7% (n=31) male and 56.3% (n=40) female patients. The mean age was 53.4 years (SD 6.5). All patients were Caucasian. The mean BMI and waist-to-hip ratio were 23.4 kg/m^2 (SD 6.0) and 0.87 (SD 0.09), respectively. Most patients were tertiary-educated adults (63.4%) without a positive family history of chronic diseases (59.2%). Nine patients (12.7 %) were current smokers and 36 patients (51.4%) had never smoked. The mean number of alcohol servings per week was 4.3 (SD 4.5). Most patients visited their dentist for a routine dental check-up twice a year (63.4%), never visited a dental hygienist (53.5%), brushed their teeth twice a day (73.2%) and performed interdental cleaning daily or more often (74.6%). The dental conditions (independent variables) and cardiovascular conditions (dependent variables) are similarly listed in table 1. Due to technical problems with the EndoPAT ™, the Reactive Hyperemia Index (RHI) of 17 patients was unknown.

Table 1. Study population

Background characteristics n=71	
Gender	
Male	31 (43.7)
Female	40 (56.3)
Age (years)	53.4 ± 6.5
BMI	23.4 ± 6.0
Waist-to-hip ratio	0.87 ± 0.09
Education	
Primary	3 (4.2)
Secondary	23 (32.4)
Tertiary	45 (63.4)
Positive family history	
None	42 (59.2)
Hypertension	20 (28.2)
Diabetes Type 1	3 (4.2)
Hypercholesterolemia	12 (16.9)
Rheumatoid arthritis	6 (8.5)
Smoking status	
Never smoked	36 (51.4)
Past smoker	34 (48.6)
Current smoker	9 (12.7)
Pack-years	4.9 ± 9.7
Alcohol servings/week	4.3 ± 4.5
Routine dental check-up	
Never	4 (5.6)
Once a year	22 (31.0)
Twice a year	45 (63.4)

Background characteristics n=71	
Dental hygienist visit	
Never	38 (53.5)
Once a year	8 (11.3)
Twice a year	16 (22.5)
≥ three times a day	9 (12.7)
Toothbrushing	
Once a day	6 (8.5)
Twice a day	52 (73.2)
≥ three times a day	13 (18.3)
Interdental cleaning	
Never	8 (11.3)
1-6 times a week	10 (14.1)
≥ daily	53 (74.6)
Dental conditions- Independent variables	
PISA	1112.2 ± 797.3
Tooth loss	5.8 ± 3.4
No periodontitis Periodontitis (stage III/IV)	30 42.3) 41 (57.7)
Plaque score	43.3 ± 25.3
Bleeding score	46.9 ± 27.0
Cardiovascular conditions - Dependent variables	
CAC	0 [10]
CAC score	
CAC = 0	45 (63.4)
CAC ≥ 1	26 (36.6)
Endothelial dysfunction (RHI)*	2.4 ± 0.8
SCORE	1.1 ± 1.4
Reynolds Risk Score	3.4 ± 3.9
Framingham Risk Score	4.3 ± 4.7
MESA Risk Score	3.0 ± 3.0

Values represent mean ± standard deviation, number of subjects (%) or median [IQR]. * RHI n=17 unknown.

We observed that 63.4% of patients had a zero CAC score, while the remainder had CAC scores ranging from 1 to 320. The CAC scores were not normally distributed, which resulted in a median of 0 (IQR 10). This prompted us to stratify all individuals into two groups. One group contained 45 patients with a CAC score zero and the other group consisted of the patients with elevated CAC scores ≥ 1 (n= 26). The characteristics of this study population were arranged per CAC group, as shown in table 2. The patients in the group with elevated CAC scores were significantly older (p=0.001). There were no significant differences in the other characteristics, health-related behavior and dental conditions between the zero CAC score and the elevated CAC score group. Endothelial dysfunction was not significantly related to elevated CAC scores (p=0.498). We found significant relations between elevated CAC scores and all cardiovascular risk prediction scores: SCORE (p=0.000), Reynolds risk score (p=0.018), Framingham risk score (0.000) and MESA risk score (p=0.000).

Table 2. Characteristics of the study population in relation to elevated CAC scores

	CAC = 0 n= 45	CAC ≥ 1 n = 26	P-value
Gender*			0.070
Male	16 (35.6 %)	15 (57.7 %)	
Female	29 (64.4 %)	11 (42.3 %)	
Age (years)**	51.6 ± 6.2	56.6 ± 5.9	0.001
BMI**	22.7 ± 7.2	24.6 ± 3.0	0.208
Waist to hip**	0.86 ± 0.06	0.90 ± 0.10	0.071
Education*			0.964
Primary	2 (4.5%)	1 (3.8%)	
Secondary	15 (33.3%)	8 (30.8%)	
Tertiary	28 (62.2%)	17 (65.4%)	
Positive family history*			0.664
None	26 (57.8%)	16 (61.5%)	0.756
Hypertension	12 (26.6%)	8 (30.8%)	0.711
Diabetes type 1	3 (6.7%)	0 (0.0%)	0.179
Hypercholesterolemia	7 (15.6%)	5 (19.2%)	0.691
Rheumatoid arthritis	6 (13.3%)	0 (0.0%)	0.052
Health related behavior			
Smoking status*			0,602
Never smoked	25 (55.6)	11 (44.0)	
Past smoker	20 (44.4)	14 (56.0)	
Current smoker	5 (11.1%)	4 (15.4%)	0.602
Pack-years**	4.5 ± 9.9	5.6 ± 9.5	0.652
Alcohol servings/week**	3.7 ± 3.6	5.3 ± 5.7	0.171
Routine dental check-up*			0.285
Never	4 (8.9)	0 (0.0)	
Once a year	13 (28.9)	9 (34.6)	
Twice a year	28 (62.2)	17 (65.4)	
Dental hygienist visit*			0.921
Never	25 (55.6)	13 (50.0)	
Once a year	5 (11.1)	3 (11.5)	
Twice a year	9 (20.0)	7 (26.9)	
≥ three times a day	6 (13.3)	3 (11.5)	
Toothbrushing*			0.778
Once a day	3 (6.7)	3 (11.5)	
Twice a day	33 (73.3)	19 (73.1)	
≥ three times a day	9 (20.0)	4 (15.4)	
Interdental cleaning*			0.370
Never	6 (13.3)	2 (7.7)	
1-6 times a week	2 (4.4)	8 (30.8)	
≥ daily	37 (82.2)	16 (61.5)	
Dental health			
PISA score*	1106.7 ± 805.1	1121.7 ± 799.4	0.940
Tooth loss*	5.3 ± 3.2	5.5 ± 3.6	0.177
Periodontal Disease stage ≥ III**	25 (55.6)	16 (61.5)	0.623
Plaque score*	41.9 ± 26.9	45.6 ± 22.6	0.558
Bleeding score*	45,5 ± 28.6	49.23 ± 24.4	0.583

	CAC = 0 n= 45	CAC ≥ 1 n = 26	P-value
Cardiovascular conditions and risk predictors			
Endothelial dysfunction (RHI) *	2.4 ± 0.8	2.3 ± 0.7	0.498
SCORE*	1.5 ± 0.7	5.7 ±3.5	0.000
Reynolds Risk Score *	2.6 ± 3.1	4.9 ± 4.8	0.018
Framingham Risk Score*	2.9 ± 3.1	6.8 ± 5.9	0.000
MESA Risk Score*	1.5 ± 0.7	5.7 ± 3.5	0.000

Values represent number of subjects (%) or mean ± standard deviation. Group differences were tested by Chi-square analysis* or independent T-Test **. Statistically significant, P-value <0.05.

Table 3A shows the univariate regression analysis between the dental and the cardiovascular conditions. The Systematic Coronary Risk Evaluation (SCORE) algorithm showed a significant relation to tooth loss (p=0.008), plaque score (p=0.039) and bleeding score (p=0.018). The Reynolds risk score was significantly associated to PISA (p=0.05), plaque score (p=0.017) and bleeding score (p=0.007). The Framingham risk score displayed a significant relation to PISA (p=0.005), plaque score (p=0.027) and bleeding score (p=0.003). In figure 1 we illustrate the association between tooth loss and the Systemic Coronary Risk Evaluation (SCORE) algorithm.

Figure 1. Association between tooth loss and SCORE

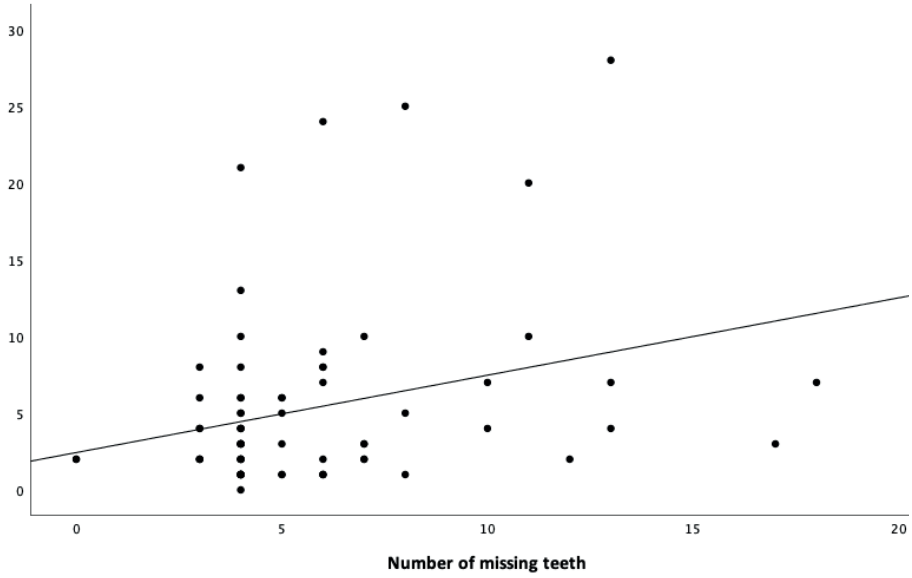

Table 3A. Univariate regression analysis

	B (SE)	OR (95%CI)	P
CAC score ≥ 1*			
PISA	0.000 (0.000)	1.000 (0.999;1.001)	0.939
Tooth loss	0.098 (0.074)	1.103 (0.955;1.274)	0.183
PD stage ≥ III	0.247 (0.502)	0.781 (0.292;2.092)	0.623
Plaque score	0.006 (0.010)	1.006 (0.987;1.025)	0.552
Bleeding score	0.005 (0.009)	1.005 (0.987;1.024)	0.577

	Unstandardized B (SE)	95% CI of B	P
RHI**			
PISA	-4.796E-5 (0.000)	0.000;0.0000	0.710
Tooth loss	0.038 (0.029)	-0.020;0.97	0.193
PD stage ≥ III	0.083 (0.211)	-0.339;0.505	0.695
Plaque score	-0.001 (.004)	-0.009;0.008	0.893
Bleeding score	3.388E-5 (0.004)	-0.007;0.007	0.993
SCORE**			
PISA	0.000 (0.000)	0.000;0.001	0.086
Tooth loss	0.131 (0.048)	0.035;0.227	0.008
PD stage ≥ III	0.559 (0.338)	-0.114;1.233	0.102
Plaque score	0.014 (0.007)	0.001;0.027	0.039
Bleeding score	0.015 (0.006)	0.003;0.027	0.018
Reynolds risk score**			
PISA	0.001 (0.001)	0.000;0.002	0.050
Tooth loss	0.237 (0.136)	-0.034;0.508	0.086
PD stage ≥ III	1.367 (0.930)	-0.488;3.221	0.146
Plaque score	0.044 (0.018)	0.008;0.079	0.017
Bleeding score	0.046 (0.016)	0.013;0.078	0.007
Framingham risk score**			
PISA	0.002 (0.001)	0.001;0.003	0.005
Tooth loss	0.233 (0.164)	-0.095;0.561	0.160
PD stage ≥ III	1.463 (1.118)	-0.795;3.667	0.204
Plaque score	0.048 (0.021)	0.006;0.091	0.027
Bleeding score	0.060 (0.020)	0.022;0.099	0.003
MESA risk score**			
PISA	0.001 (0.000)	0.000;0.002	0.157
Tooth loss	0.063 (0.107)	-0.151;0.276	0.560
PD stage ≥ III	0.594 (0.725)	-0.852;2.041	0.415
Plaque score	0.018 (0.014)	-0.010;0.047	0.196
Bleeding score	0.021 (0.013)	-0.005;0.047	0.113

Univariate binary logistic regression analysis* and univariate linear regression analyses** were performed to assess the association between each independent variable and the dependent variables. Statistically significant, P-value <0.05.

In the multivariate regression analysis, we adjusted for the most relevant confounders: gender, age, BMI, waist-to-hip ratio, education, alcohol and smoking (Table 3B). None of the dental conditions were significantly related to the cardiovascular risk predictors after correcting for the confounders in the multivariate regression analysis.

Table 3B. Multivariate regression analysis

	Unstandardized B (SE)	95% CI	P
SCORE			
Tooth loss	0.032 (0.041)	-0.050;0.114	0.437
Plaque score	0.004 (0.005)	-0.005;0.014	0.375
Bleeding score	0.004 (0.005)	-0.006;0.014	0.396
Reynolds risk score			
PISA	-2.804E-5 (0.001)	-0.001;0.001	0.963
Plaque score	0.023 (0.016)	-0.008;0.055	0.144
Bleeding score	0.019 (0.016)	-0.013;0.051	0.241
Framingham risk score			
PISA	0.000 (0.001)	-0.001;0.001	0.676
Plaque score	0.019 (0.015)	-0.012;0.50	0.231
Bleeding score	0.023 (0.016)	-0.009;0.054	0.153

Multivariate regression analysis, adjusted by the most relevant confounders: gender, age, BMI, waist to hip ratio, education, alcohol and smoking. Statistically significant, P-value <0.0

Discussion

In this prospective cross-sectional study, we found significant relations between tooth loss, dental plaque and bleeding scores and the CVD risk predictors: SCORE, Reynolds risk score and Framingham risk score. However, when adjusting for confounders (gender, age, BMI, waist-to-hip ratio, education, alcohol and smoking) this association was no longer significant. Similarly, the significant relations between the Periodontal Inflamed Surface Area (PISA) score and the Framingham and Reynolds CVD risk predictors were no longer significant after correcting for these confounders. We did not find significant associations between the presence and extent of coronary calcification, as investigated by the CAC score, and periodontitis or dental health. Nor did we find significant associations between endothelial dysfunction and periodontitis or poor dental health.

This study is the third to have explored the possible correlation between Coronary Artery Calcium (CAC) scores and dental health, especially periodontitis. The Atherosclerosis Risk in Communities (ARIC) study was the first study that used the CAC score to investigate the association with periodontitis. The ARIC study included healthy patients and patients with known (chronic) diseases (excepting clinically recognized CVD), more than 20 years ago. The mean interval between the dental examinations and CAC score was 2.4 years (range: 0.9 to 4.3 years).Its results suggested that periodontitis is not strongly associated with CAC.[29] The second study used the Coronary Artery Calcification in Type 1 diabetes patients (CACTI study), conducted in 2003. Periodontitis was self-reported by an unvalidated questionnaire and clinical dental examination was not performed. The researchers concluded that in patients with Type 1 diabetes, periodontal disease duration was significantly related to CAC progression, but this was not the case in subjects without diabetes.[30] Taking above mentioned into account, we are the first study that included exclusively, asymptomatic healthy patients from the dental practice. The CAC score was investigated approximately 2 weeks after the full-mouth examination, performed by a periodontist. Due to the explorative nature of this study, a proper power-analysis was not applicable and the Medical Ethics Committee approved this presented sample size. In retrospect, enlargement of the study population would have strengthened the current study.

The CAC score is proven as a strong biomarker for cardiovascular atherosclerotic diseases. The absence of CAC (CAC=0) provides the strongest ‘negative risk factor’ compared to traditional end novel cardiovascular biomarkers, especially in asymptomatic patients.[31] All the patients in our study population were asymptomatic, and most of them had a zero CAC score. Instead, the presence of coronary calcification on a cardiac CT scan is a late phenomenon. Endothelial dysfunction has been recognized as an important indicator of more early-stage atherosclerosis. A possible clinical scenario could be to use the Reactive Hyperemia Index (RHI) as a first screening, and if this indicates vascular disease, CAC scores could be calculated to

add one more prognostic indicator.[24] In this study, there was no significant relation between periodontitis or dental health and endothelial dysfunction. A limitation is that, due to technical problems with the EndoPAT™, the RHI of 17 patients was unknown.

Previous pathophysiological evidence points to the possible common inflammatory background between periodontitis and atherosclerosis.[8] Another dental pathological condition is apical periodontitis. In this situation, there is a chronic inflammation around the apex of a tooth, caused by bacterial invasion of the pulp and root canal, most often as a result of untreated dental carries. These peri-apical lesions contain bacteria that can be translocated throughout the body and lodge in various organs and atherosclerotic plaques.[32] Nevertheless, there are only a few studies that have suggested a link between CVD and chronic apical periodontitis. [33], [34] In this study, the presence of peri-apical lesions was not taken into account, but we focused on the most common dental pathology: periodontitis.

Periodontitis and CVD are complex inflammatory diseases with genetic and epigenetic factors that interact with lifestyle and environmental factors such as smoking, nutrition and stress. Both diseases are considerably influenced by similar multilevel interactions between metabolic and immune systems. The relatively recent realization that obesity affects the immune system and promotes inflammation may provide a plausible mechanism for the observed overlap between periodontitis and cardiovascular diseases.[35] Like obesity, the other components of metabolic syndrome (dyslipidemia, diabetes/hyperglycemia, and hypertension) are also linked to periodontitis through a number of pathomechanisms.[36] Moreover, the shared genetic basis of periodontitis and CVDs has recently been demonstrated.[37] It seems that the overall profile of a typical periodontitis patients is similar to the profile of a CVD patient, and vice versa.

We included patients who visited a specialized dental clinic for periodontology. It must be taken into account that these patients are not fully representative of the general population. This selection bias is a limitation of this study.

To conclude, based on this study, periodontitis is associated to a higher risk for cardiovascular morbidity and mortality, but is not an independent risk factor. Considering the findings of this study and previous studies, it is still increasingly important to bridge the once-wide gap between dentistry and general medicine to identify patients at risk for cardiovascular diseases in an earlier stage.

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CHAPTER 5

The effect of periodontal treatment on the reactive hyperemia index
A one-year follow-up pilot study

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Abstract

Background

Periodontitis is a chronic multifactorial inflammatory disease of the supportive tissues of the teeth. In more recent years, remarkable epidemiological and pathophysiological associations between periodontitis and cardiovascular disease (CVD) have been presented. Whether or not treatment of periodontitis is valuable for primary or secondary prevention of cardiovascular disease, has not yet been fully established. In this practice-based pilot study we focused on primary prevention of cardiovascular disease, by investigating the effect of periodontal treatment on the earliest detectable stage of CVD; endothelial dysfunction.

Methods

Otherwise healthy periodontitis and non-periodontitis participants 45-70 years of age were included in the study. One year after completing periodontal (non-surgical and surgical) treatment of the periodontitis patients and one year after inclusion of the controls, all baseline measurements were repeated. Full-mouth examinations were performed by a periodontist to determine their Periodontal Inflamed Surface Area (PISA) score and other dental parameters. To assess the cardiovascular conditions, endothelial function through the reactive hyperemia index (RHI) assessed by the EndoPAT ™, and several physical and biochemical parameters were measured.

Results

21 patients with diagnosed, untreated periodontitis and 21 participants without periodontitis were included in this follow-up study. After periodontal therapy in the periodontitis patients, the PISA reduced significantly. The RHI did not show a significant improvement after treatment of the periodontitis patients (-0.1 ± 0.8, p=0.524). Similarly, other secondary cardiovascular outcome measurements, hsCRP, total cholesterol, HDL cholesterol, triglycerides, HbA1c and systolic blood pressure did not improve significantly after periodontal treatment. Controls did not show any significant changes in the RHI, in other CVD parameters and in the PISA after one-year follow-up.

Conclusions

In this practice-based pilot study, periodontal treatment did not improve the endothelial function in otherwise healthy adults with periodontitis. Future studies are needed to be of larger size and could focus on periodontitis patients with co-morbidities to investigate whether periodontal treatment has secondary preventive effect on endothelial function and other CVD parameters.

Introduction

Periodontitis is a chronic multifactorial inflammatory disease of the supportive tissues of the teeth.[1] It is the most common oral disease, and the sixth most common human disease, affecting 30-50% and approximately 10% of the global adult population in its most severe form.[2], [3] Periodontitis starts with localized inflammation of the gingiva that is initiated by bacteria in the dental plaque. As a result of inflammation, the tissues surrounding the tooth are infiltrated by neutrophils, macrophages and activated lymphocytes. The subsequent state is periodontitis, with progressive loss of alveolar bone and tooth attachment and ultimately tooth loss. Bacteria and cytokines from the periodontal inflammatory lesions are dispersed throughout the body and acute phase reactants (C-reactive protein [CRP], fibrinogen) are produced[4]. Although bacteria are necessary for periodontitis to take place, a susceptible host is also needed. Consequently, several risk factors for periodontitis have been established, including smoking, diabetes mellitus, socio-economic position, psychosocial factors and genetic predispositions.[5] Interestingly, some genetic risk variants for cardiovascular diseases show overlap with identified genetic variants of periodontitis.[6]

The first study that found positive epidemiological evidence for the association between periodontitis and atherosclerosis was in 1989 by Mattila *et al*..[7] In more recent years, remarkable pathological and epidemiological associations between these two diseases have been presented, though without final conclusions.[8]–[11] The explanation of the association between periodontitis and CVD generally fall into two categories: (a) microbial mechanisms, which through vascular invasion may locally affect the development of the atheroma lesions; and (b) inflammatory and immunologic mechanisms that directly influence the pathobiology of the atheroma lesion.[12] Most pathophysiological links between these two diseases are based on the possible common inflammatory background between periodontitis and CVD. [13], [14]

The possible link between CVD and periodontitis is of great public health importance because of the high prevalence of both diseases and the potential impact on public health if risk modification or therapeutic opportunities could be identified. Whether or not treatment of periodontitis is valuable for primary or secondary prevention of cardiovascular disease, have not yet been fully established. The majority of the intervention trials, aimed to study this purpose, has examined the effect of periodontal treatment on markers of systemic inflammation, focusing primarily on the role of common inflammatory pathways.[15]

Endothelial dysfunction has been recognized as the critical junction between CVD risk factors and clinical disease, and is the earliest detectable stage of CVD.[16] Tonetti *et al*. sought to assess the effect on intensive periodontal treatment on endothelial function measured by Flow-Mediated dilatation (FMD) of the brachial artery. The FMD was greater, and thus

improved, in the intensive-treatment group than in the control-treatment group 60 days after therapy (absolute difference 0.9%; 95% CI, 0.1 to 1.7; $P = 0.02$) and 180 days after therapy (difference, 2.0%; 95% CI, 1.2 to 2.8; $P < 0.001$). The degree of improvement was associated with improvement in measures of periodontal disease.[17] More recent studies did not find clinical evidence for a positive effect on endothelial function after periodontal therapy, and therefore a possible cause-to-effect relationship remains controversial.[18], [19]

In this pilot study we focused on possible primary prevention of CVD in a practice-based setting. Accordingly, the primary aim of this study was to determine the effect of periodontal treatment on endothelial function as assessed by the reactive hyperemia index (RHI) in otherwise healthy patients with periodontitis. Secondary aims were to investigate the effect of periodontal treatment on other parameters, including high sensitive C-reactive Protein (hsCRP), (systolic) blood pressure, cholesterol (HDL, LDL and total), triglycerides and glycosylated hemoglobin (HbA1c).

Material and Methods

Study design

This dental-practice based clinical intervention study was a one-year follow-up, of our prospective cross-sectional study as published previously.[20] The study was approved by the Medical Ethics Committee, Isala Academy, Zwolle, the Netherlands (NL43083.075.13) and has been registered in the ISRCTN trial registry with study ID ISRCTN55656827. All participants provided written consent for participation at baseline and at the time of follow-up. This study was performed in accordance with the Declaration of Helsinki guidelines for human research, 1964, and amended in 2013 (64th World Medical Association General Assembly, Fortaleza, Brazil). Data were collected, analyzed and interpreted by the authors.

Participants

We included periodontitis patients and control subjects, between 45 and 69 years of age, without known systemic diseases and with at least 10 teeth. At baseline, all subjects were included in our previously published prospective cross-sectional study.[20] The periodontitis patients received periodontal treatment after the baseline measurements. One year after completing periodontal treatment, these patients were recruited for participation in this follow-up study. The control group was also recruited for participation in this follow-up study, one year after the baseline measurements. All mentioned measurements below were performed at baseline and after one year of follow-up.

Measures of dental health

All participants underwent a full-mouth periodontal examination performed by two trained periodontists at the Practice for Periodontology Zwolle (PPZ). Inter observer agreement of these two periodontists was examined by comparing individual measurements of ten random participants. The statistically determined intraclass correlation coefficient of 0.81 represented an excellent reliability according to Fleiss.[21] Periodontitis was initially diagnosed and staged according to the consensus report of the World Workshop on the classification of periodontal and peri-implant diseases and conditions.[22] The Periodontal Inflamed Surface Area (PISA) score was applied. This scoring tool calculated the amount of inflamed periodontal tissue in square millimeters and quantified the total inflammatory burden resulting from periodontitis.[23] The PISA score was calculated after extensive periodontal examination, including periodontal probing pocket depth (PD), plaque score and bleeding on probing (BOP). All measurements were performed on all teeth, on six sites per tooth using a manual periodontal standard probe. Rather than presenting mean pocket probing results and bleeding on probing scores, we regarded it essential for the current study to use the PISA score because it is the best integrative and overall score for quantifying the inflammatory burden posed by periodontitis. Moreover, it can be easily and broadly interpreted and applied by clinicians as well as patients.

Measures of general health

All participants completed questionnaires to collect data on their medical history, perceived health, parental history, lifestyle, socio-economic status and oral hygiene.

At least two weeks after the periodontal examination, the participants were examined by a trained nurse at the Department of Cardiology of the Isala hospital, Zwolle. Physical examinations were performed, and blood pressure (BP), heart rate (HR), body mass index (BMI), waist-to-hip ratio (WHR), and electrocardiogram measurements (ECG) were obtained. Venous blood was collected to determine levels of high sensitive C-reactive Protein (hsCRP[mg/L]), total cholesterol (mmol/L), HDL-cholesterol (mmol/L), LDL-cholesterol (mmol/L), triglycerides (mmol/L), estimated Glomerular Filtration Rate (eGFR ml/min/1.73m^2) and glycosylated hemoglobin (HbA1c [%]).

Measures of cardiovascular condition

As primary outcome we performed an endothelial function assessment by the EndoPAT ™ (Itmar Medical, Israel), based on noninvasive Peripheral Arterial Tone (PAT) signal technology measuring endothelium-mediated changes in vascular tone using bio-sensors placed on the fingertips. This endothelial function assessment is validated versus the invasive gold standard (intracoronary infusion of acetylcholine). It is a short and operator independent endothelial dysfunction test, that is easy practice or office based performable.[24] The final result of the EndoPAT ™ is the Reactive Hyperemia Index (RHI), which is a ratio of the post-to-pre occlusion PAT amplitude of the tested arm, divided by the post- to pre- occlusion ratio of the control arm. A RHI score of 1.67 and below correlates to endothelial dysfunction.[25], [26]

Periodontal treatment

All periodontitis patients received periodontal treatment, including training in oral hygiene, and counselling on control of risk factors (e.g. smoking, alcohol usage and overweight/obesity). Supra- and subgingival bacterial plaque and calculus was removed by comprehensive, meticulous periodontal scaling and root planning performed by an experienced dental hygienist in the specialized periodontal practice using local anesthesia. When the non-surgical treatment had insufficient effect on the pocket depth and bleeding score, or when residual pockets deeper than 5 mm were still present, surgical treatment by a periodontist was incorporated. Surgical treatment consisted of procedures to reduce or eliminate periodontal pockets and create an acceptable gingival contour that facilitates oral hygiene and periodontal maintenance. Selective teeth that could not be saved were extracted. Finishing procedures included posttreatment evaluation with review and reinforcement of daily oral hygiene when appropriate. Thereafter, patients were enrolled in a periodontal maintenance program of 3-4 times a year performed by the dental hygienist of the specialized periodontal practice.

Controls

The controls without periodontitis were educated in oral hygiene and were counselled on control of risk factors for periodontitis (e.g. stress and smoking). The dental health was maintained by their own dental hygienist 1-2 times a year.

Statistics

Descriptive statistics (mean ± standard deviations (SD), Median (Q1-Q3) or numbers (%) of subjects) were used to present patient characteristics and results, depending on the distribution. Between group differences were tested by the Fisher's exact test or Chi-square test for categorical variables and independent T-Tests for quantitative variables. Change scores were calculated by subtracting old values from new values. Hence a negative value indicates a lower follow up value, i.e. decrease. Within group differences/changes were tested using the McNemar test for categorical variables and the paired T-test or the Wilcoxon signed ranks test for quantitative variables, depending on the distribution of change. The significance level was set at a p-value of 0.05. All analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). Figures were created using RStudio version 1.4.1717 (R version 4.0.3).

Results

Inclusion

At baseline, 27 patients with untreated periodontitis and 27 controls without periodontitis were included. Of these 54 participants, 12 subjects (6 periodontitis patients and 6 control subjects) were lost to follow-up, because of refusing to participate. Thus 21 periodontitis patients and 21 non-periodontitis controls were included in this follow-up study. There was no significant difference at baseline between the age, gender, BMI, PISA score and endothelial function between the total 54 participants and the 42 participants included for follow-up.

Participants

The study population consisted of 40.5% (n=17) male and 59.5% (n=25) female participants. The mean age was 54.1 ± 6.3 years. All participants were Caucasian. Fifty percent (n= 21) of these participants were tertiary educated, and 42.9% (n=18) were secondary educated. Forty-one percent of the participants had a positive family history for CVD. The characteristics of these 42 participants are presented in Table 1. There was no significant change in periodontitis risk factors between baseline and follow-up (BMI p=0.791, waist to hip ratio p=0.259, smoking p=1.000, alcohol intake p=0.798).

Table 1. Characteristics of the study population

	Total	**Periodontitis**	**Control**
	n=42	**n=21**	**n=21**
Age	54.1 ± 6.3	55.4 ± 7.1	52.9 ± 5.4
Gender			
Male	17 (40.5)	10 (47.6)	7 (33.3)
Female	25 (59.5)	11 (52.4)	14 (66.7)
BMI	24.3 ± 3.2	25.4 ± 3.8	23.2 ± 1.8
Waist to hip ratio	0.9 (0.8–0.9)	0.9 (0.2–1.0)	0.9 (0.8–0.9)
Smoking	4 (9.5)	4 (19.0)	0 (0.0)
Alcohol servings /week*	4.0 ± 4.1	2.2 ± 2.7	5.4 ± 4.5
Education			
Primary	3 (7.1)	2 (9.5)	1 (4.8)
Secondary	18 (42.9)	8 (38.1)	10 (47.6)
Tertiary	21(50.0)	11 (52.4)	10 (47.6)
Positive family history for CVD	17 (40.5)	7 (33.3)	10 (47.6)

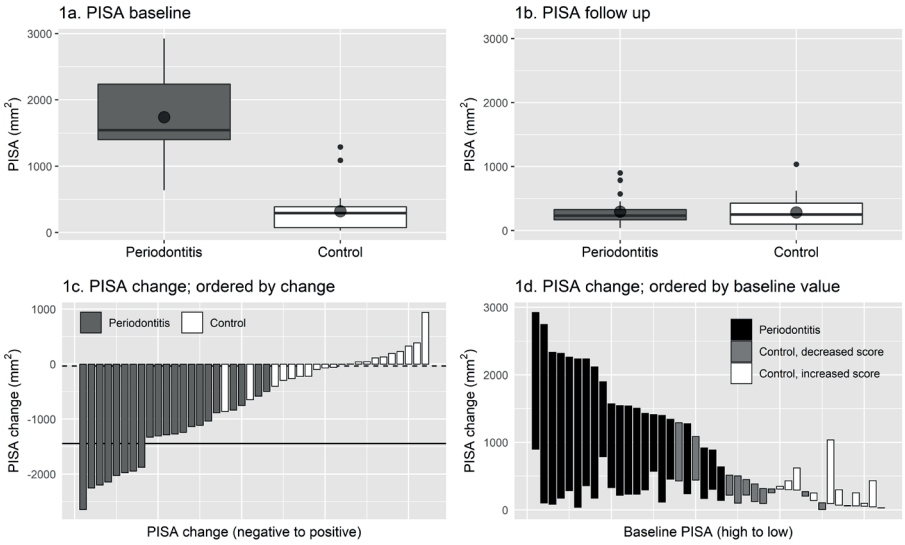
*Alcohol servings /week: 8 missing
Values represent mean ± standard deviation, number of subjects (%), or median (Q1-Q3).
There were no statistically significant differences between the periodontitis and the controls.

Dental health

Figure 1 and table 2 show baseline values, 1-year follow-up values and changes in dental health. The inflammatory burden of the periodontitis patients improved significantly after periodontal treatment: one year after completing the periodontal treatment, the mean decrease of the PISA score was -1444.8 mm^2 ± 612.4 ($p < 0.001$). In the control group, mean decrease of the PISA score was -35.6 mm^2 ($p=0.670$). Figure 1c visualizes an association of baseline PISA score with PISA change.

The mean decrease of the plaque score was -47.8% ± 19.1 and the mean decrease of the bleeding score was -54.4% ± 19.9. Due to selective tooth extraction, as part of the periodontal treatment, the number of teeth also significantly decreased ($p=0.017$). The dental health of the control group did not significantly change during the follow-up period.

Figure 1. Changes in PISA score



Figures 1a and 1b are boxplots showing the distribution of PISA score at baseline and at follow up, respectively. Point shapes indicate sample means. Figures 1c and 1d are waterfall plots showing the change of PISA score while ordered by the extent of PISA change and ordered by baseline PISA score, respectively. In figure 1c the solid line indicates mean PISA change for periodontitis patients and the dashed line indicates mean change for controls.

Table 2. Changes in dental health

	Baseline	Follow-Up	Change	p-value
All				
PISA score	1030.1 ± 868.0	290.0 ± 226.0	-740.2 ± 872.2	<0.001
Teeth	28.0 (26.0–28.0)	27.0 (25.0–28.0)	0.0 (-0.3–0.0)	0.005
Plaque score	40.6 ± 28.1	15.1 ± 9.9	-25.5 ± 30.1	<0.001
Bleeding score	43.8 ± 31.1	15.1 ± 9.8	-28.7± 33.2	<0.001
Periodontitis				
PISA score	1740.7 ± 612.5	295.9 ± 221.6	-1444.8 ± 612.4	<0.001
Teeth	28.0 (24.0–28.5)	26.0 (22.0–28.0)	0.0 (-2.5–0.0)	0.017
Plaque score	60.6 ± 19.4	12.8 ± 7.2	-47.8 ± 19.1	<0.001
Bleeding score	67.6 ± 18.4	13.2 ± 9.0	-54.4 ± 19.9	<0.001
Control				
PISA score	319.6 ± 330.3	284.0 ± 235.7	-35.6 ± 376.6	0.670
Teeth	28.0 (26.0–28.0)	28.0 (26.0–28.0)	0.0 (0.0–0.0)	0.102
Plaque score	20.7 ± 20.0	17.4 ± 11.7	-3.3 ± 21.2	0.485
Bleeding score	20.0 ± 21.2	17.1 ± 10.3	-2.9 ± 21.6	0.544

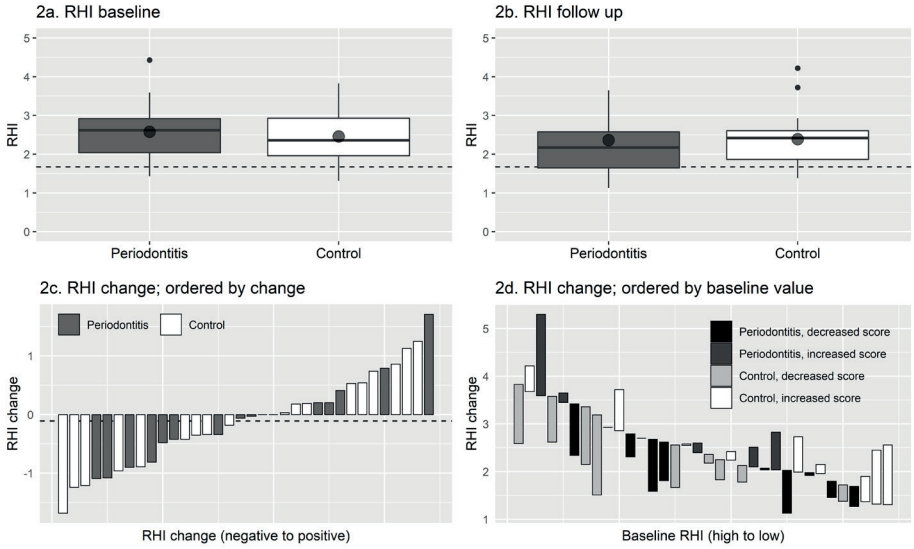
Values represent mean ± standard deviation, or median (Q1-Q3). Group differences were tested by a paired T-test or Wilcoxon signed rank test.

Cardiovascular health

Data on cardiovascular conditions at baseline and after the follow-up period of the periodontitis and controls are shown in Table 3. Endothelial function, the primary outcome, expressed as RHI, did not show a significant improvement after treatment of the periodontitis patients (RHI -0.1 ± 0.8, p=0.524). Figure 2A and 2B illustrates the minor changes in endothelial function, expressed by the RHI, observed from baseline to 1 year after completing periodontal therapy. In Figure 2C and 2D we present waterfall plots to demonstrate the change of the endothelial function ordered by the extent of the RHI change and ordered by the RHI at baseline. Due to technical problems with the EndoPAT ™, the RHI of 8 subjects (6 periodontitis patients and 2 control subjects) was unknown. Similarly, all other secondary cardiovascular outcome measurements, hsCRP, total cholesterol, HDL cholesterol, triglycerides, HbA1c and systolic blood pressure did not significantly improve after periodontal therapy.

After the 1-year follow-up period of the controls, none of the cardiovascular variables significantly changed.

Figure 2. Changes in endothelial function (RHI)



Figures 2a and 2b are boxplots showing the distribution of RHI at baseline and at follow up, respectively Point shapes indicate sample means. The dashed line presents RHI cut-off score of 1.67 that correlates to endothelial dysfunction. Figures 1c and 1d are waterfall plots showing the change of RHI while ordered by the extent of RHI change and ordered by baseline RHI, respectively. In figure 1c the dotted line indicates mean RHI change for periodontitis patients.

Table 3. Changes in cardiovascular conditions

	Baseline	Follow-Up	Change	p-value
All				
Endothelial function (RHI)	2.5 ± 0.7	2.4 ± 0.9	-0.1 ± 0.8	0.424
hsCRP	1.1 (0.6–2.3)	0.75 (0.0–1.9)	-0.3 (-0.6–0.3)	0.123
Total cholesterol	5.4 ± 0.8	5.3 ± 0.8	-0.1 ± 0.7	0.350
HDL cholesterol	1.7 ± 0.5	1.7 ± 0.4	0.0 ± 0.2	0.705
LDL cholesterol	3.3 ± 0.9	3.2 ± 0.8	-0.1 ± 0.6	0.173
Triglycerides	1.0 (0.7–1.2)	0.9 (0.7–1.1)	0.0 (-0.2–0.1)	0.634
HbA1c	36.1 ± 2.7	36.3 ± 2.6	0.3 ± 1.3	0.195
Systolic BP	126.3 ± 15.2	123.2 ± 11.3	-3.1 ± 13.4	0.113
Periodontitis				
Endothelial function (RHI)	2.5 ± 0.6	2.4 ± 1.0	-0.1 ± 0.8	0.524
hsCRP	1.2 (0.6–2.8)	1.0 (0.0–2.5)	-0.3 (-0.7–0.5)	0.339
Total cholesterol	5.2 ± 0.8	5.1 ± 0.7	-0.1 ± 0.4	0.185
HDL cholesterol	1.6 ± 0.5	1.6 ± 0.4	-0.0 ± 0.2	0.787
LDL cholesterol	3.1 ± 0.8	3.0 ± 0.7	-0.2 ± 0.4	0.101
Triglycerides	1.0 (0.6–1.4)	0.9 (0.8–1.4)	0.0 (-0.2–0.4)	0.490
HbA1c	36.9 ± 2.6	36.8 ± 2.5	-0.0 ± 1.2	0.858
Systolic BP	129.3 ± 16.6	125.4 ± 11.0	-3.9 ± 13.8	0.208
Control				
Endothelial function (RHI)	2.5 ± 0.8	2.4 ± 0.7	-0.1 ± 0.8	0.623
hsCRP	0.9 (0.6–1.7)	0.6 (0.0–1.4)	-0.2 (-0.4–0.1)	0.169
Total cholesterol	5.7 ± 0.8	5.6 ± 0.9	-0.1 ± 0.9	0.693
HDL cholesterol	1.7 ± 0.4	1.8 ± 0.4	0.0 ± 0.2	0.438
LDL cholesterol	3.5 ± 0.9	3.4 ± 0.9	-0.1 ± 0.7	0.578
Triglycerides	0.9 (0.7–1.2)	0.8 (0.7–1.1)	-0.1(-0.2–0.0)	0.099
HbA1c	35.3 ± 2.6	35.9 ± 2.6	0.6 ± 1.3	0.062
Systolic BP	123.3 ± 13.4	121.1 ± 11.4	-2.3 ± 11.1	0.357

RHI missing at follow-up: 8 (6 periodontitis and 2 control)
Values represent mean ± standard deviation, or median (Q1-Q3). Group differences were tested by a paired T-test or Wilcoxon signed rank test.

Association between periodontal improvement and cardiovascular health

Table 4 emphasizes that there were no significant differences in change of the cardiovascular variables after treatment of the periodontitis patients compared to the control group. Figure 3 illustrates that there was no significant association between the decrease of the PISA score, periodontal improvement, and the endothelial function, expressed in RHI.

Table 4. Differences in cardiovascular change between periodontitis patients and controls

	Change periodontitis	Change control	Difference	p-value
Endothelial function (RHI)	-0.1 ± 0.8	-0.1 ± 0.8	-0.1 (NA)	0.960
hsCRP	-0.3 (-0.7–0.5)	-0.2 (-0.4–0.1)	-0.1 (NA)	0.880
Total cholesterol	-0.1 ± 0.4	-0.1 ± 0.9	-0.0 (-0.5–0.4)	0.839
HDL cholesterol	-0.0 ± 0.2	0.0 ± 0.2	-0.1 (-0.2–0.1)	0.452
LDL cholesterol	-0.2 ± 0.4	-0.1 ± 0.7	-0.1 (-0.4–0.3)	0.689
Triglycerides	0.0 (-0.2-0.4)	-0.1(-0.2-0.0)	-0.1 (NA)	0.232
HbA1c	-0.0 ± 1.2	0.6 ± 1.3	-0.6 (-1.4–0.2)	0.121
Systolic BP	-3.9 ± 13.8	-2.3 ± 11.1	-1.6 (-9.4–6.2)	0.677

Values represent mean ± standard deviation, or median (Q1-Q3). Group differences were tested by an independent T-test or Mann-Whitney U test.

Figure 3. Association between periodontal improvement and endothelial function.

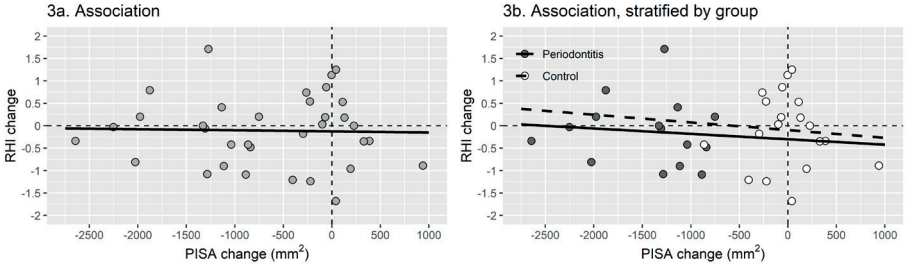


Figure 3. (a) Association between PISA change and RHI change for the total study population (R^2 linear = 6.549E-4). (b) Association between PISA change and RHI change stratified by periodontitis patients (R^2 linear = 0.008) and control group (R^2 linear = 0.006) (b).

Discussion

The current clinical intervention follow-up study aimed to investigate the effect of periodontal treatment on endothelial function, in otherwise healthy adults. The clinical results showed that significant decrease of the inflammatory burden after periodontal treatment did not improve the endothelial function, as measured by the RHI with the EndoPAT ™, or other cardiovascular parameters after one-year follow-up.

Positive effects of periodontal treatment on endothelial function and other cardiovascular parameters that were demonstrated in previous intervention studies, focused more on patients already suffering from CVD. Correspondingly, levels of hsCRP of the included untreated periodontitis patients were somewhat lower than previously studies reported.[27], [28] In this study we focused on otherwise healthy adults, without a history of CVD. Only 5 participants suffered from endothelial dysfunction at baseline. The mean baseline endothelial function, expressed in RHI, in our study population was higher than the RHI cut-off score of 1.67 that correlates to endothelial dysfunction. Probably, because the RHI at baseline was well within normal limits, a positive effect of periodontal treatment could not be expected. Besides, we used the EndoPAT ™ device for measuring endothelial function. This device might not be sensitive enough to detect small differences in endothelial function.

Some systematic reviews support the positive effects of periodontal treatment on CVD risk parameters, especially 6 months after the treatment. This effect extinguished after a follow-up time of 12 months.[29] We have chosen consciously for a follow-up period of one year, because intervention studies with a 1-year follow-up time are scarce. Furthermore, an even more extended follow-up time, with cardiovascular events as hard endpoint, is needed to confirm or reject the causal relation between periodontitis and CVD. However, these kind of studies are challenging, due to methodological, financial and most important, ethical considerations.[30]

Despite numerous publications investigating the association between periodontitis and CVD, there is still no consensus whether periodontitis plays a pathophysiological role in CVD.[8] Ever since the publication of the first studies indicating an association, this topic received substantial professional and public interest. Consequently, this subject generated debates between researchers, caused wide-scale media coverage and prompted involved professional organizations to issue official statements. It remains essential to understand the quality of the underlying literature to be able to perform a critical appraisal. Regrettably, we must be aware of poor reporting and misinforming, concerning the clinical trials evaluating the effect of periodontal treatment on CVD.[31]

A limitation of this study is the relatively small study group. Although the effect of periodontal treatment on endothelial function in periodontitis patients has been suggested in the literature, this has rarely been investigated in exclusively, otherwise healthy periodontitis

patients. We are the first study that investigated asymptomatic healthy periodontitis patients from the dental practice, using the endothelial function assessment by the EndoPAT. We focused on this specific patient population, to investigate whether the apparently otherwise healthy periodontitis patients in the dental practice are possibly at risk for a CVD event in the future and whether periodontal treatment can reduce such risk for a CVD event. Due to this explorative nature of the study, a proper power-analysis was not applicable. In retrospect, enlargement of the study population would have strengthened the current study. Besides, we included patients who visited a specialized dental clinic for periodontology. It must be taken into account that these patients may not fully representative of the general population.

With the current pilot study, we have attempted to add further knowledge to the once-wide gap between dentistry and general medicine, aimed to identify patients at risk for CVD in an earlier stage. In conclusion, periodontitis and CVD are complex inflammatory diseases with shared modifiable and non-modifiable risk factors. Periodontal treatment as primary or secondary prevention of CVD could be focused on direct control of periodontitis and changing modifiable risk factors of both. In this study, we did not find an improvement of endothelial function or other cardiovascular parameters after highly effective periodontal treatment including nonsurgical and surgical therapy. Future studies are needed to be a larger size and could focus on periodontitis patients with co-morbidities, whether periodontal treatment has secondary preventive effect on endothelial function and other CVD parameters.

Acknowledgements

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PART II

Dental status as a window to COVID-19

CHAPTER 6

Alveolar bone loss and tooth loss are associated with COVID-19 severity but are not independent risk factors. An explorative study

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2022

Abstract

Purpose

This study explores the association between alveolar bone loss, tooth loss and severity of COVID-19.

Materials and Methods

In this retrospective cohort study, we included patients with confirmed COVID-19 who have had a dental panoramic radiograph within a maximum period of 5 years, providing information about alveolar bone loss and tooth loss. The severity of COVID-19 was determined based on the WHO clinical progression scale: (1) Mild/Ambulatory; (2) Moderate/Hospitalized; (3) Severe/Intensive care unit (ICU) or death.

Results

1730 patients were identified with COVID-19 from until October 31, 2020 in the Isala Hospital. Of these patients, 389 ever visited the OMFS department. 133 patients have had an orthopantomograph within a maximum period of 5 years and were included for analysis. The results showed a significant association between alveolar bone loss and COVID-19 severity (p=0.028). Patients with alveolar bone loss had 5.6 times higher odds to be admitted to ICU or died, compared to ambulatory patients (OR: 5.60; 95%CI: 1.21; 25.99; P=0.028). More tooth loss was significantly associated with COVID-19 severity (p=0.047). Per tooth lost, patients had 4.2% higher odds for severe than mild COVID-19 (OR: 1.04; 95%CI: 1.00; 1.09; P=0.047) and 6.0% higher odds for severe than moderate COVID-19 (OR: 1.06; 95%CI: 1.01; 1.11; P=0.017). When adjusting for confounders in multivariate analyses, the significant associations of COVID-19 with alveolar bone loss and tooth loss were no longer present.

Conclusion

In this retrospective explorative pilot study, alveolar bone loss and tooth loss are associated with the severity of COVID-19, however they are not independent risk factors. The current study could contribute to the design of further studies on the relationship between oral health and COVID-19.

Introduction

At the end of 2019, the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in China. The later designated coronavirus disease 2019 (COVID-19) rapidly developed in a worldwide pandemic.[1] Indication of risk factors for severe disease, hospital admission and death became crucial. Age is one of the highest risk factors for morbidity and mortality due to infection with SARS-CoV-2.[2] Additionally, cardiovascular disease, male sex, chronic kidney disease, and obesity are associated with hospital admission and unfavorable outcomes.[3]–[5]

Several epidemiological and pathological associations between poor oral health and systemic diseases have been reported. Periodontal disease and its ultimate sequela tooth loss, are associated with an increased risk of non-communicable diseases (NCDs), including cardiovascular diseases, cancer, diabetes, Alzheimer's disease and respiratory tract infection. [6], [7] In addition, several studies have demonstrated that bacteria, microbial products and cytokines translocated from oral inflammatory conditions, cause exacerbations of inflammatory reactions in distant organs, for example increased vascular damage in atherosclerosis and other cardiovascular processes.[8] However, causality has never been demonstrated and associations are probably the result of shared risk factors and comorbidities.

There are sufficient data demonstrating that coexisting conditions in patients with COVID-19 influence clinical outcomes. Potential risk factors could help clinicians to identify patients with poor prognosis at an early stage. It has been hypothesized that poor oral health is associated with the severity of the clinical progression of COVID-19.[9] Marouf et al. recently found an association between periodontal disease and the severity of COVID-19 in hospitalized patients. [10] Tooth loss is the ultimate state of dental pathology and poor oral health. The current retrospective cohort study explored the association between periodontal disease and tooth loss, and the course and outcome of COVID-19.

Material and Methods

Study oversight

This retrospective cohort study was approved by the Medical Ethics Committee, Isala Academy, Zwolle, the Netherlands (200710). Requirement for informed consent was waived. This study was done in accordance with the Declaration of Helsinki guidelines for human research, 1964, and amended in 2013 (64th World Medical Association General Assembly, Fortaleza, Brazil). Data were collected, interpreted and analyzed by the authors.

We included hospitalized patients and outpatients from the Isala Hospital (Zwolle, the Netherlands) with confirmed COVID-19 who visited the Department of Oral and Maxillofacial Surgery (OMFS) and who have had a dental panoramic radiograph (Orthopantomograph – OPG), obtained up to a maximum of 5 years until the end of the current study. The patient inclusion cutoff for the study was October 31, 2020. Confirmed COVID-19 was defined as a positive SARS-CoV-2 real-time reverse transcription polymerase chain reaction (rRT-PCR) on swab material, sputum or bronchoalveolar lavage samples.

The electronic health records provided information about age, sex, body mass index (BMI), diabetes mellitus, cardiovascular diseases, chronic kidney disease and smoking. When a medical condition such as diabetes mellitus or cardiovascular disease was not mentioned in a patient file, but the corresponding medication was documented (e.g. metformin and/or insulin, statins and antihypertensive drugs), the patient was scored positively for that disorder. For all patients, the BMI was calculated based on the height and weight noted in the health records (maximum retrieval period 365 days).

COVID-19

The course and outcome of COVID-19 was determined based on the WHO Clinical progression Scale: (1) Mild disease: Ambulatory; (2) Moderate disease: Hospitalized; (3) Severe disease: intensive care unit (ICU) admission or death.[11]

Oral health

Each dental panoramic radiograph was scored by three investigators blinded for the COVID-19 severity. Periodontal disease was defined when alveolar bone loss (ABL) ≥1/3 of the root length was detected at two or more non-adjacent teeth, according the recent Classification of Periodontal and Peri-implant Diseases.[12] Periodontal disease (PD) was scored as present of absent. Alveolar bone loss related to periodontal-endodontic lesions, cracked and fractured roots, caries, restorative factors and impacted third molars was not scored. The number of teeth present was measured by counting all teeth visible on the OPG, including third molars and radices relictae; dental implants, pontics of fixed partial dentures and prosthetic dentures

were not counted as teeth. The number of missing teeth was calculated by subtracting the number of present teeth from the expected total of 32 teeth. Patients were noted as dentate or edentulous.

Statistics

Descriptive statistics (mean ± standard deviations [SD] or numbers [%] of subjects) were used to present patient characteristics and dental findings. Group differences were tested by one-way analysis of variance (ANOVA) for quantitative variables or Chi-square analysis for categorical variables. P-value of 0.5 was set at the significance level. Univariate multinomial logistic regression analyses were used to assess the unadjusted associations between periodontal disease, the number of teeth lost and the outcome of COVID-19. Next, we screened important confounders between COVID-19 and the two independent variables, respectively, by performing separate multinomial logistic regression analyses. In each multinomial logistic regression analysis, only confounder was included with the independent variable. Next, the confounders with $P<0.05$ were included in the subsequent fully adjusted multivariate models. Statistical analyses were performed using IBM SPSS Statistics 26 software (SPSS Inc., Chicago, IL, USA).

Results

1730 patients were identified with COVID-19 from March 1st until October 31, 2020 in the Isala Hospital. Of these patients, 389 ever visited the Department of Oral and Maxillofacial Surgery (OMFS). We retrieved 157 patients with confirmed COVID-19 who visited the OMFS department within a maximum period of 5 years. 133 patients have had a dental panoramic radiograph (Orthopantomograph – OPG) (Figure 1). In 115 (86.5%) patients the OPG was assessed before COVID-19 and in 18 (13.5%) patients COVID-19 was before their visit to the OMFS department. The mean intermediate period between COVID-19 and the OPG was 695 days (SD 543).

Figure 1. Inclusion flowchart

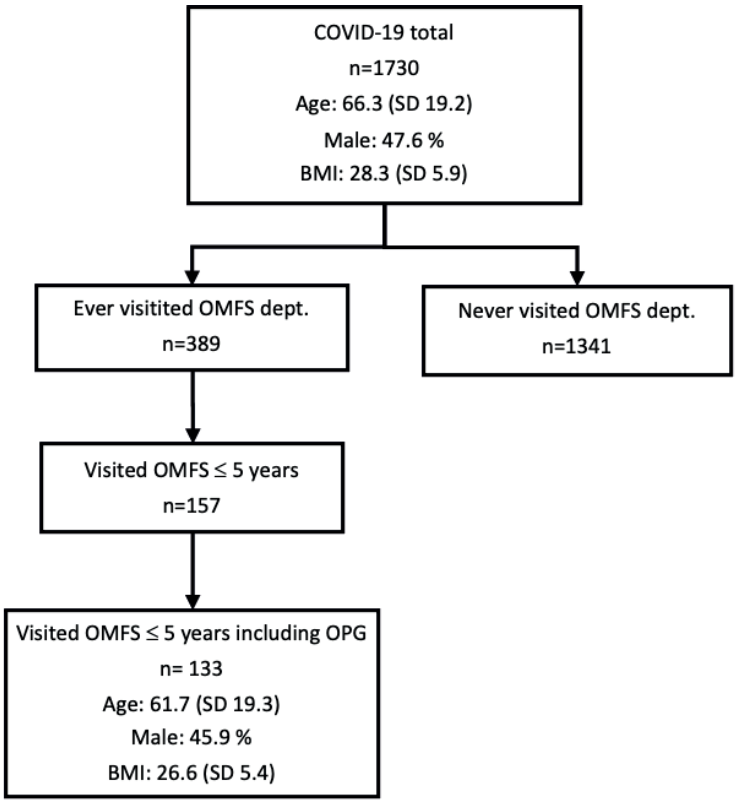

Figure 1 displays the age, sex and BMI of the total COVID-19 population and the included patients. There was no significant difference between the sex (p=0.688). Compared to the total COVID-19 population, the 133 included patients were significant younger (p=0.008) and the BMI was significant lower (p=0.007). All patient characteristics of these included 133 patients are presented in Table 1. The population of this study consisted of 46% (n=61) male patients. The mean age was 61.7 years (SD 19.3). The BMI of 25 patients was unknown, for 108 patients the mean BMI was 26.6 kg/m^2 (SD 5.4). 15.8 percent (n=21) of the patients were having diabetes mellitus (2 unknown), 48.9% (n=65) of the patients suffered from cardiovascular disease, and 9.8% (n=13) of the patients suffered from chronic kidney disease. The smoking status of 7 patients was unknown, while among 126 patients, 12 (9%) were current smokers.

Table 1. Background characteristics of patients with COVID-19.

	Total COVID19 n = 133	Mild; Ambulatory n = 82	Moderate; Hospitalized n=30	Severe; ICU/Death n=21	p-value
Age (years)	61.7±19.3	57.3±21.0	63.5±13.9	75.9±10.6	0.000*
Male sex	61 (45.9)	29 (35.4)	16 (53.3)	16 (76.2)	0.001**
Body Mass Index	26.6±5.4	26.3±5.3	27.1±3.4	27.9±4.7	0.420*
Diabetes mellitus	21 (15.8)	9 (11.1)	5 (16.7)	7 (33.3)	0.013**
Cardiovascular disease	65 (48.9)	34 (41.5)	14 (46.7)	17 (81.0)	0.003**
Chronic kidney disease	13 (9.8)	6 (7.3)	2 (6.7)	5 (23.8)	0.055**
Smoking	12 (9.0)	6 (7.3)	4 (13.3)	2 (9.5)	0.013**

Values represent number of subjects (%) or mean ± standard deviation. Group differences were tested by * one-way analysis of variance (ANOVA) or ** Chi-square analysis (linear by linear). Statistically significant, P-value <0.05.

In Table 2 we present dental pathology findings of the COVID 19 patients based on the OPG assessments. Within the group of dentate patients (n=92), 14.1% was scored positive for periodontal disease based on the alveolar bone loss. The results showed a significant association between alveolar bone loss and the progression categories of COVID-19 (P=0.028). Patients with alveolar bone loss had 5.6 times higher odds to be admitted to the ICU or die, compared to the ambulatory COVID-19 patients (OR: 5.60; 95%CI: 1.21; 25.99; P=0.028).

Table 2. Dental pathologies of patients with COVID-19.

	Total COVID19 n = 133	Mild; Ambulatory n = 82	Moderate; Hospitalized n=30	Severe; ICU/Death n=21	p-value
Periodontal disease◆	13 (14.1)	5 (9.3)	4 (15.4)	4 (57.1)	0.028**
Number of teeth	16.0±12.2	16.4±12.7	18.8±10.4	10.3±11.0	0.043*
Edentulous	41 (30.8)	28 (34.1)	4 (13.3)	9 (42.9)	0.961**

Values represent number of subjects (%) or mean ± standard deviation.
◆determined as alveolar bone loss ≥1/3 of the root length for dentate patients (N=92)
Group differences were tested by * one-way analysis of variance (ANOVA) or ** Chi-square analysis (linear-by-linear). Statistically significant, P-value <0.05.

The number of teeth was significantly associated with the severity of COVID-19 based on one-way ANOVA (p=0.043). The patients with more missing teeth, were more likely to have a severe clinical outcome (ICU admission or death) than a mild or moderate outcome. With the number of teeth decreasing by one unit, the patients had 4.2% higher odds to have severe COVID-19 than mild clinical outcome (OR: 1.04; 95%CI: 1.00; 1.09; P=0.047). Also, with the number of teeth decreasing by one unit, the patients had 6.0% higher odds for a severe clinical outcome than a moderate clinical outcome (OR: 1.06; 95%CI: 1.01; 1.11; P=0.017). We observed 9 out of 16 (42.9%) edentulous patients in the group of patients with severe clinical outcome, compared to 34.1% and 13.3% in the mild and moderate categories, but there was no significant association between edentulousness and COVID-19.

To further explore whether the dental pathologies could be independent risk factors for the severity of COVID-19, we first screened for the confounders. Separate multinomial logistic regression analyses performed identified that age, male sex, diabetes mellitus, cardiovascular diseases, chronic kidney disease and smoking were the significant confounders between periodontal disease and the progression of COVID-19. In the multivariate analysis adjusting for these confounders, alveolar bone loss was not significantly associated with the severity of COVID-19 when the mild clinical outcome was compared with severe outcome (OR: 3.332; 95%CI: 0.394; 28.148; p=0.269) and when the moderate clinical outcome was compared with the severe outcome (OR: 3.214; 95%CI: 0.354; 29.197; p=0.300).

Another set of separate multinomial logistic regression analysis identified that age, male sex and cardiovascular diseases were the significant confounders between the number of teeth and the clinical outcome of COVID-19. When adjusting for age, male sex and cardiovascular disease in the multivariate model, tooth loss was not significantly associated with the clinical outcome of COVID-19 (P=0.453 when mild clinical outcome was compared with severe outcome, and P=0.263 when moderate clinical outcome was compared with severe outcome).

Discussion

This retrospective, cohort study was initiated to explore the association between parameters of poor oral health and the severity of COVID-19. We observed a statistically significant association between the COVID-19 severity with alveolar bone loss and the most obvious and definitive dental pathological event: tooth loss. However, when adjusted for the well-known risk factors of COVID-19, these dental parameters were not identified as independent risk factors for the course and outcome of COVID-19 in our study population.

We included rRT-PCR-confirmed COVID-19 patients who visited the Department of Oral and Maxillofacial Surgery (OMFS) and who have had a dental panoramic radiograph obtained up to a maximum of 5 years. This population was younger and showed a lower BMI than the total population of confirmed COVID-19 patients. This possible selection bias is corrected with the multivariate logistic regression analysis, where we included these confounders.

Tooth loss is the ultimate state of dental pathology. Beyond middle age, most tooth loss is the “end point” of periodontal disease. This prolonged state of chronic inflammation with increased levels of C-Reactive Protein (CRP) is a proven risk factor for non-communicable diseases (NCDs) which are also associated with unfavorable outcomes of COVID-19.[3], [7] However, most tooth loss before middle age is caused by dental caries. Dental caries is a disease with a multifactorial etiology; consumption of dietary carbohydrates is one of the most important etiological factors. Carbohydrate intake is also associated with increased risk for infection and mortality rates of COVID-19 across the world.[13], [14] Besides, tooth loss might affect dietary intake and nutritional status among adults and thereby affecting the general condition and strength to fight COVID-19.[14], [15] Above all, tooth loss might cause harmful health benefits and has been considered to impact quality of life.[16]

In the current study we used a dental panoramic radiograph (Orthopantomograph – OPG) to measure the alveolar bone loss due to periodontal disease and to count the number of present teeth. Regarding the alveolar bone loss, for reasons of a possible degree of uncertainty of minor alveolar bone loss to be observed on OPG, we identified subjects having severe periodontal disease with at least 2 non-adjacent teeth with bone loss ≥1/3 of the root length according to the current classification.[12] Since only radiographical and no clinical information was obtained to determine the periodontal disease, no assumptions could be made on the activity of the dental pathology. Periodontal disease can be in an active, in a chronic or in a remission state.

The number of teeth present, and correspondingly the tooth loss, is an easily accessible marker and can be determined by most; the general practitioner, the dentist or even the patient itself. We assumed that loss of teeth was a result of dental pathology with dental

caries and periodontal disease as leading causes. This should be carefully interpreted since in some cases a tooth may have been lost due to non-pathological causes such as orthodontic treatment, dental trauma and agenesis. However, the incidence of those events is low.

The maximum time allowed between the OPG and the COVID-19 diagnosis was five years. However, the average time between these two radiographic assessments was less than 2 years (695 days). We are aware that there is the possibility that the number of teeth, could have decreased in the course of the time between COVID-19 and the radiographic status. We assumed that the progression of the studied dental pathologies, is a rather slow processes and changes within this timeframe will not be large. For this study we deemed the maximum of 5 years acceptable. Nevertheless, more periodontal disease and less teeth present than currently scored at the actual time of COVID-19, most likely would have strengthened the current findings.

Another limitation of our study is the sample size. It would have been superior to have more dental records or OPGs of the confirmed COVID-19 patients. However, the current study was set up as a retrospective explorative study to assess whether the most clear dental events, tooth loss and alveolar bone loss, were associated with COVID-19 severity. Obviously due to the retrospective design, it was not possible to include more patients with available dental records or OPGs during this rapidly developing pandemic.

Conclusion

This study provides suggestive evidence that the severity of COVID-19 is associated with alveolar bone loss and the ultimate “hard” endpoint of dental pathology, i.e. tooth loss. However, when adjusted for the well-known risk factors of COVID-19, these dental parameters were not identified as independent risk factors for the course and outcome of COVID-19 in our study population. The current clinical investigation should be considered as an explorative pilot study that could contribute to the design of further studies on the relationship between poor oral health and the severity of COVID-19. Nevertheless, the current findings add to the wealth of research showing the relationship between oral health and general health, which is probably the result of shared risk factors and underlying conditions. Tooth loss is as an easily and quick accessible proxy for a severe COVID-19 course of disease, hospital admission and death, which is crucial during this worldwide pandemic. Dental professionals must be aware that patients with extensive tooth loss may have increased risk for more severe clinical progression and outcome of COVID-19.

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CHAPTER 7

Development and external validation of prediction models for critical outcomes of unvaccinated COVID-19 patients based on demographics, medical conditions and dental status

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Abstract

Background

Multiple prediction models were developed for critical outcomes of COVID-19. However, prediction models using predictors which can be easily obtained in clinical practice and on dental status are scarce. The study aimed to develop and externally validate prediction models for critical outcomes of COVID-19 for unvaccinated adult patients in hospital setting based on demographics, medical conditions, and dental status.

Methods

A total of 285 and 352 patients from two hospitals in the Netherlands were retrospectively included as derivation and validation cohorts. Demographics, medical conditions, and dental status were considered potential predictors. The critical outcomes (death and ICU admission) were considered endpoints. Logistic regression analyses were used to develop two models: for death alone and for critical outcomes. The performance and clinical values of the models were determined in both cohorts.

Results

Age, number of teeth, chronic kidney disease, hypertension, diabetes, and chronic obstructive pulmonary diseases were the important predictors. The models showed good to excellent calibration with observed: expected (O:E) ratios of 0.98 and 1.00, and discrimination with area under the curve (AUC) values of 0.85 and 0.79, based on the derivation cohort. In the validation cohort, the models showed good to excellent discrimination with AUC values of 0.85 and 0.78, but an overestimation in calibration with O:E ratios of 0.65 and 0.67.

Conclusions

The performance of the models was acceptable in both derivation and validation cohorts. Number of teeth was an additive important predictor of critical outcomes of COVID-19. It is an easy applicable tool in hospitals for risk stratification of COVID-19 prognosis.

Introduction

The novel coronavirus disease 2019 (COVID-19) pandemic has presented an important and urgent threat to global health since its outbreak in December 2019. COVID-19 does not only affect the respiratory tract, but it also affects other organs in human body i.e., lungs, liver, kidney, heart, vessels.[1] Respiratory failure and acute respiratory distress syndrome (ARDS) are the most common serious complications of COVID-19 infection.[2] The relative excess deaths (excess mortality) from all causes in 2020 was up to 20% in England, Greece, and Switzerland, and up to 30% in Italy and Spain.[3] The in-hospital mortality of COVID-19 was reported to be 17.1% based on 33 studies from 13,398 patients [4], and it was 2.9 times higher than that of influenza based on the French national administrative database.[5] It was reported that 26% of the COVID-19 patients were admitted to the intensive care unit (ICU) with severe status and 31% of the patients who were admitted to ICU died based on 37 studies from 24,983 patients.[6] While the COVID-19 vaccines have provided strong protection against serious illness, hospitalization, and death [7,8] around 40% of the worldwide population is still unvaccinated until 25th January 2022.[9]

There are several risk factors on patients` demographic characteristics and underlying medical conditions which were shown to be associated with the critical outcomes of COVID-19.[10] In addition, poor oral health, in particular periodontitis, was also shown to be associated with the critical outcomes of COVID-19. [11-13]. Kamel et al. [12] showed a significantly inverse moderate association between oral health and COVID-19 severity (r=-0.512) in 208 Egyptian COVID-19 patients based on a cross-sectional study. Marouf et al. [14] showed that COVID-19 patients with periodontitis had 8 times higher odds of death and 3.5 times higher odds of ICU admission than those without periodontitis in hospitalized patients based on a case-control study. This may be because periodontal disease could enhance cytokine release via pathogenic microflora, expression of multiple viral receptors, bacterial superinfection, and aspiration of periodontal pathogens.[15] The increased production of pro-inflammatory cytokine, which is referred to as cytokine storm, is the foremost cause of the adverse events of COVID-19.[15]

The high contagiousness, high ICU admission rate, and high mortality of COVID-19 have led to tremendous increases in the demand for hospital beds and shortage of medical equipment. Therefore, there is an urgent need for a pragmatic risk stratification tool that allows for the early identification of the COVID-19 patients who are likely to be at highest risk of ICU admission and death.[16] This can help clinicians and policymakers make evidence-based decisions on the management of COVID-19 patients and optimize resource allocation. Recently, multiple prediction models have been developed for the prediction of the prognosis of COVID-19 patients.[17,18] Those prediction models varied in their predictors and performance of the models. A large number of prediction models carry difficulties in their application for the rapid risk stratification of general COVID-19 patients at their first intake in hospitals. This is

because some predictors cannot be easily obtained without professional devices or lab tests, such as C reactive protein, peripheral oxygen saturation, urea level, white cell count, and lymphocytes.[17] Furthermore, many prediction models showed moderate performance in aspects of discrimination and calibration, and beard no benefit to clinical decision-making. [17] In addition, the oral condition and the dental status were never considered potential predictors in the previously developed models.

Therefore, the present study aimed to develop and externally validate prediction models for the early and rapid stratification of critical outcomes of COVID-19 patients using predictors which can be easily obtained in clinical practice, including patients` demographic characteristics, medical conditions, and dental status.

Methods

Participants

We included consecutive hospitalized patients and outpatients from the Isala Hospital (Zwolle, the Netherlands) who were diagnosed with COVID-19 between January 2020 and May 2021, and visited the Department of Oral and Maxillofacial Surgery (OMFS) of the hospital up to five years until the COVID-19 diagnosis as the derivation cohort for the development of the prediction models. We included the consecutive hospitalized patients and outpatients from Northwest Clinics (Alkmaar, the Netherlands) who were diagnosed with COVID-19 between January 2020 and July 2021, and visited the Department of OMFS of the hospital up to five years until the COVID-19 diagnosis as the validation cohort. All the included patients were adults and unvaccinated. Confirmed COVID-19 was defined as a positive SARS-CoV-2 real-time reverse transcription-polymerase chain reaction (rRT-PCR) on swab material, sputum, or bronchoalveolar lavage samples.

This study was approved by the Medical Ethics Committee, Isala Academy, Zwolle, the Netherlands (200710), and taken over by Northwest Academy, Alkmaar, the Netherlands (L021-054). Requirement for informed consent was waived. This study was done in accordance with the Declaration of Helsinki guidelines for human research, 1964, and amended in 2013 (64th World Medical Association General Assembly, Fortaleza, Brazil).

Potential predictors

The potential predictors included patients` demographic characteristics, medical conditions, and dental status.

The demographic characteristics included sex, age at the diagnosis of COVID-19, and body mass index (BMI). The BMI was calculated based on the height and weight noted in the most recent patients` electronic health records with the maximum retrieval period of one year.

The medical conditions included diabetes mellitus (DM), chronic obstructive pulmonary diseases (COPD), cardiovascular diseases (CVD), obstructive sleep apnea (OSA), chronic kidney disease (CKD), hypertension (HT), and hypercholesterolemia (HCL). The medical conditions were dichotomized as presence or absence. The information on medical conditions was first collected from electronic health records. When a medical condition was not mentioned in a patient file, but the corresponding medication was documented (e.g. metformin and/or insulin, statins, and antihypertensive drugs), the patient was considered to have such medical disorder. Besides, the smoking status of patients was also collected from the patients` health records. The patients were classified into current smokers, previous smokers, and non-smokers.

Dental status included the number of remaining natural teeth excluding the third molars ranging from 0 to 28 and number of implants the patients received, which is commonly used as proxy clinical indicators for dental health, such as periodontal diseases and dental caries. [19,20]The predictors on dental status were collected based on the orthopantomogram (OPG) taken within the past five years. The number of remaining natural teeth was measured by counting all teeth visible on the OPG including radices relicta. Pontics of fixed partial dentures and prosthetic dentures were not counted as teeth. In the analysis, the number of remaining teeth was classified into 0 teeth, 1-19 teeth, and 20-28 teeth based on the commonly used cutoffs.[21]

Outcome (endpoint)

The endpoint of the study was the presence or absence of the critical outcomes of COVID-19. The course and outcome of the COVID-19 was classified into (1) ambulatory; (2) hospitalized; (3) ICU admission or death, based on the WHO Clinical Progression Scale.[22] In the study, the critical outcomes were defined as ICU admission or death, while the non-critical outcomes were defined as ambulatory or hospitalized without ICU admission.

Statistics

Missing data

The multiple imputation technique was used for the missing values in both the derivation and validation datasets. We created m=30 imputed datasets with 10 iterations and used predictive mean matching (PMM) for imputing the missing values. All the potential predictors and the outcome variables were included in the imputation model. In the imputation model, number of remaining teeth and number of implants were included as continuous variables.

Development of the models

Screening of potential predictors and modeling

Multicollinearity of the potential predictors was tested using the variance inflation factor (VIF). When a VIF value of a predictor was higher than 5, collinearity was considered present and the predictor was excluded from the following analysis.[23]

Two prediction models were developed for the prediction of death only and for the prediction of the critical outcome due to COVID-19 (death or ICU admission combined). For each outcome variable, the univariate association between each potential predictor and the outcome variable was first assessed with univariate logistic regression analyses. Predictors with a p-value of ≤0.15 were selected for the subsequent multivariate analyses. Multivariate binary logistic regression analysis with backward selection (predictors with p >0.15 were removed from the models) was used to further assess the association of potential predictors with the outcome in the multivariate setting, and to develop the prediction model.

Shrinkage factor

To prevent the overfitting of the current model that has been developed from a derivation dataset and for over-optimism of a model applied in similar future populations, the regression coefficients of the predictors in the models were multiplied by a shrinkage factor.[24,25] A shrinkage factor ranges from 0 to 1 and was derived using the bootstrapping procedure with 100 bootstrap samples.

Calibration

Calibration is defined as the agreement between predicted outcomes and observed outcomes.[26] The calibration of the models was assessed by plotting the predicted individual outcomes against the observed actual outcomes. For this, study members were grouped into deciles based on their predicted probabilities for the outcomes. The prevalence of the outcome events within each decile represents the observed probability. The mean of the individual predicted probabilities within each decile represents the predicted probability. In the calibration plot, the

observed and predicted probabilities were compared across the range of predicted risk. The overall observed: expected ratio (O:E ratio) was also used for the assessment of the overall calibration of the models.[26] The O:E ratio was obtained by dividing the prevalence of the outcomes (observed) with the mean of individual predicted probabilities of the outcomes (expected) within the cohort.[27] An O:E ratio <1 indicates an overestimation of the models, while an O:E ratio >1 indicates an underestimation of the models.[28] An O:E ratio between 0.8 and 1.2 indicates that the calibration of the model is acceptable.[28] The calibration of the multivariate models was also assessed using the Hosmer-Lemeshow goodness-of-fit statistic test (HL test). A p-value of >0.10 in the HL test indicates that the model fits the observed data.[29]

Discrimination

Discrimination is defined as the ability to differentiate between those with and those without the outcome event.[26] The area under the receiver-operating characteristic curves (AUC) was used to assess the discrimination of the models.[29] An AUC of 0.70 to 0.80 indicates that the discrimination of the models is acceptable, while an AUC of ≥0.80 indicates that the discrimination of the models is excellent to outstanding.[31]

The optimal cutoff for the predicted probability of the models was defined as the predicted probability with the maximum sum of sensitivity and specificity in the receiver-operating characteristic curve (ROC).

Clinical values

Clinical values of the models at the optimal cutoff for predicted probability were assessed using prevalence (prior probability) and posterior probabilities of the outcome events. The posterior probability was defined as positive predictive value (PPV) and negative predictive value (NPV). PPV was the number of the patients with the outcome events in reality in patients with the outcome events as predicted by the models. NPV was the number of patients without the outcome events in reality in patients without the outcome events as predicted by the models. The (added) predictive value of the models for ruling in an increased risk of the outcome events was defined as the PPV minus prevalence, while that for ruling out an increased risk of the outcome events was defined as NPV minus complement of prevalence.

Scoring system

A clinical prediction rule for the outcome events was developed to provide an estimate for individual patients of their absolute risk of developing the outcome events. For the final multivariate binary logistic regression models, the individual probability (P) of the outcome events is predicted with the following formula:

$$P = 1 - 1/[1 + \exp(\text{constant} + \beta 1X1 + \ldots + \beta iXi)]$$

Where β is the shrunken regression coefficient of a predictor in the models.

To facilitate the calculation of the predicted probabilities of the outcome events in individual patients separately, the multivariate logistic regression models were converted to a score chart. The score of each included predictor in the score chart was produced by the shrunken regression coefficients being divided by the smallest regression coefficient of the predictors and subsequently rounded. Line charts were then developed to help determine the predicted probability of the outcome events.

External validation of the models

To assess the general applicability of the models, the derived prediction models were externally validated based on the validation cohort. In the validation cohort, the predicted probability for the outcome event of each patient was calculated based on the developed prediction models in the derived cohort mentioned above. The performance of the models in the validation cohort was also assessed in aspects of calibration and discrimination. The prevalence, PPV, NPV, and the added predictive values of the models in the validation cohort were calculated based on the cutoff for predicted probability established in the validation cohort.

All the statistical procedures mentioned above were performed based on the imputed datasets via SPSS software 27.0 (IBM, New York, USA) and R software 4.0.4 (R Development Core Team, Vienna, Austria). The discrimination, calibration, added values, and scoring system of the models were all assessed based on the shrunken regression coefficients.

Results

A total of 285 unvaccinated patients with the diagnosis of COVID-19 (138 females and 147 males) were enrolled in the study as the derivation cohort (Figure 1). The mean age ± standard deviation (SD) of the patients was 61.1 ± 17.0 years. The mean age ± SD of male patients was 63.8 ± 14.6 years, while that of female patients was 58.2 ± 18.9 years. Of the 285 patients, 48 patients (17%) died due to COVID-19, and 62 patients (22%) developed the critical outcomes (i.e. ICU admission or death) due to COVID-19. A total of 352 unvaccinated patients (199 females and 153 males) were enrolled in the study as the validation cohort (Figure 1). The mean age ± SD of the patients was 55.4 ± 21.8 years. The mean age ± SD of male patients was 60.7 ± 20.2 years, while that of female patients was 51.3 ± 22.2 years. Of the 352 patients, 39 patients (11%) died, and 52 patients (15%) developed the critical outcomes. Table 1 presents the main characteristics and the information on the missing values of the potential predictors and the outcome variables of both derivation and validation cohorts. Table 2 presents the distribution of the potential predictors on the outcome variables based on the multiple imputations of both derivation and validation cohorts.

Figure 1. Flow diagram of patient inclusion

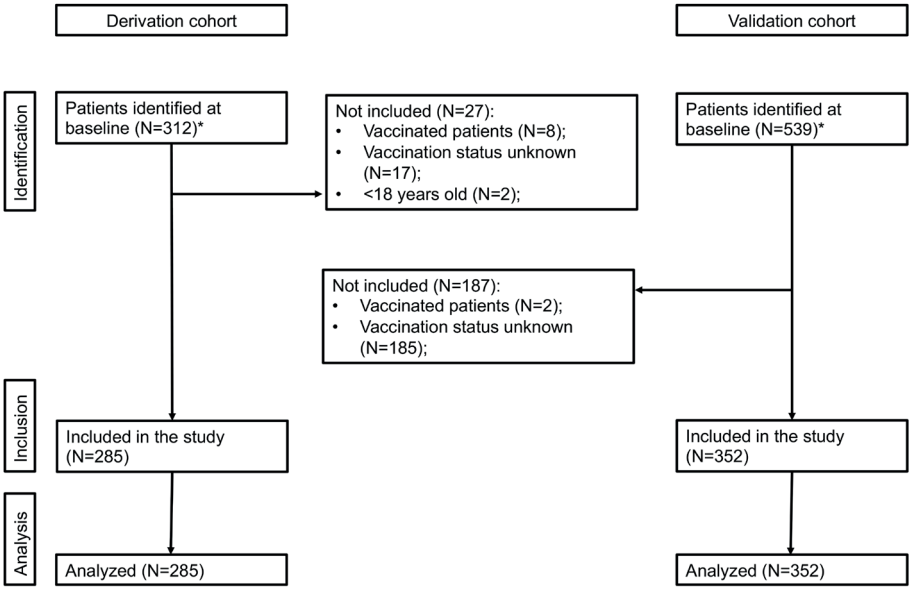

Figure 1. Flow diagram of patients inclusion (*, the patients identified at baseline were the hospitalized patients and outpatients who were diagnosed as COVID-19 between January 2020 and May 2021 in the derivation cohort and between January 2020 and July 2021 in the validation cohort and visited the Department of Oral and Maxillofacial Surgery of the hospitals up to five years until the COVID-19 diagnosis)

Table 1. Characteristics of the predictors and the outcomes

Variables	Derivation cohort (n=285)	Validation cohort (n=352)
	Mean (SD) / No. of patients (%)	Mean (SD) / No. of patients (%)
Predictors		
Sex		
Female	138 (48%)	199 (57%)
Male	147 (52%)	153 (43%)
Missing	0 (0%)	0 (0%)
Age	61.1 (17.0)	55.4 (21.8)
Missing	0 (0%)	0 (0%)
Number of teeth (excluding third molars)	15.9 (11.6)	20.0 (10.4)
Missing	72 (25%)	115 (33%)
Number of implants	0.5 (1.2)	0.3 (0.9)
Missing	74 (26%)	114 (32%)
BMI	28.0 (5.4)	26.9 (5.0)
Missing	98 (34%)	156 (44%)
Smoking		
Non-smokers	111 (54%)	124 (55%)
Previous smokers	80 (39%)	82 (37%)
Present smokers	16 (8%)	18 (8%)
Missing	78 (27%)	128 (36%)
DM		
No	237 (83%)	296 (85%)
Yes	48 (17%)	52 (15%)
Missing	0 (0%)	4 (1%)
COPD		
No	247 (87%)	285 (82%)
Yes	38 (13%)	63 (18%)
Missing	0 (0%)	4 (1%)
CVD		
No	231 (81%)	243 (70%)
Yes	54 (19%)	105 (30%)
Missing	0 (0%)	4 (1%)
OSA		
No	243 (85%)	328 (94%)
Yes	42(15%)	20 (6%)
Missing	0 (0%)	4 (1%)
CKD		
No	261 (92%)	319 (92%)
Yes	24 (8%)	29 (8%)
Missing	0 (0%)	4 (1%)
HT		
No	187 (66%)	230 (66%)
Yes	98 (34%)	118 (34%)
Missing	0 (0%)	4 (1%)
HCL		
No	256 (90%)	283 (82%)
Yes	29 (10%)	63 (18%)
Missing	0 (0%)	6 (2%)

Variables	Derivation cohort (n=285)	Validation cohort (n=352)
	Mean (SD) / No. of patients (%)	Mean (SD) / No. of patients (%)
Outcomes		
Death		
No	237 (83%)	313 (89%)
Yes	48 (17%)	39 (11%)
Missing	0 (0%)	0 (0%)
Critical outcomes		
No	223 (78%)	300 (85%)
Yes (ICU or death)	62 (22%)	52 (15%)
Missing	0 (0%)	0 (0%)

BMI, body mass index; DM, diabetes mellitus; COPD, chronic obstructive pulmonary diseases; CVD, cardiovascular diseases; OSA, obstructive sleep apnea; CKD, chronic kidney diseases; HT, hypertension; HCL, hypercholesterolemia; ICU, intensive care unit

Table 2. Distribution of predictors on the outcomes in derivation cohort (N=285) and validation cohort (N=352) based on the multiple imputation

Potential predictors	Derivation cohort					Validation cohort				
	No. of participants (%)	Death due to COVID-19		Critical outcomes of COVID-19		No. of participants (%)	Death due to COVID-19		Critical outcomes of COVID-19	
		No (n=237)	Yes (n=48)	No (n=223)	Yes (n=62)		No (n=313)	Yes (n=39)	No (n=300)	Yes (n=52)
Sex										
Female	138 (48%)	119	19	115	23	199 (57%)	190	9	188	11
Male	147 (52%)	118	29	108	39	153 (43%)	123	30	112	41
Age	61.1 ± 17.0	58.0 ± 16.6	76.1 ± 9.3	58.0 ± 16.9	72.2 ± 12.0	55.4 ± 21.8	52.7 ± 21.3	77.0 ± 10.7	52.4 ± 21.7	72.8 ± 12.6
Number of teeth										
0 teeth	87 (31%)	60	27	55	32	55 (16%)	40	15	36	19
1-19 teeth	43 (15%)	30	13	29	15	47 (13%)	34	12	31	16
20-28 teeth	155 (54%)	147	8	139	16	250 (71%)	239	12	233	18
Presence of implants										
No	219 (77%)	180	39	169	50	309 (88%)	279	30	268	40
Yes	66 (23%)	57	9	54	12	44 (12%)	34	9	32	12
BMI	27.8 ± 5.3	27.7 ± 5.2	28.3 ± 5.5	27.5 ± 5.2	28.7 ± 5.7	26.7 ± 5.0	26.8 ± 5.0	26.0 ± 4.7	26.7 ± 5.0	26.8 ± 4.6
Smoking										
Non-smokers	155 (54%)	137	18	127	28	200 (57%)	179	21	175	25
Previous smokers	106 (37%)	80	26	77	29	115 (33%)	98	16	90	25
Present smokers	24 (9%)	20	4	19	5	38 (11%)	36	2	35	2
DM										
No	237 (83%)	206	31	197	40	300 (85%)	275	25	266	34
Yes	48 (17%)	31	17	26	22	53 (15%)	39	14	35	18
COPD										
No	247 (87%)	210	37	199	48	289 (82%)	263	26	253	36
Yes	38 (13%)	27	11	24	14	64 (18%)	51	13	48	16
CVD										
No	231 (81%)	200	31	188	43	247 (70%)	237	10	226	21
Yes	54 (19%)	37	17	35	19	105 (30%)	76	29	74	31
OSAS										
No	243 (85%)	204	39	190	53	332 (94%)	298	34	285	47
Yes	42 (15%)	33	9	33	9	20 (6%)	15	5	15	5

Potential predictors	Derivation cohort					Validation cohort				
	No. of participants (%)	Death due to COVID-19		Critical outcomes of COVID-19		No. of participants (%)	Death due to COVID-19		Critical outcomes of COVID-19	
		No (n=237)	Yes (n=48)	No (n=223)	Yes (n=62)		No (n=313)	Yes (n=39)	No (n=300)	Yes (n=52)
CKD										
No	261 (92%)	222	39	210	51	323 (92%)	298	25	286	37
Yes	24 (8%)	15	9	13	11	29 (8%)	15	14	14	15
HT										
No	187 (66%)	172	15	165	22	234 (66%)	223	11	215	19
Yes	98 (34%)	65	33	58	40	118 (34%)	90	28	85	33
HCL										
No	256 (90%)	217	39	206	50	288 (82%)	266	21	254	33
Yes	29 (10%)	20	9	17	12	64 (18%)	47	18	46	19

BMI, body mass index; DM, diabetes mellitus; COPD, chronic obstructive pulmonary diseases; CVD, cardiovascular diseases; OSA, obstructive sleep apnea; CKD, chronic kidney diseases; HT, hypertension; HCL, hypercholesterolemia; critical outcome, death or ICU admission

The VIF values of all the predictors were lower than 5. Therefore, the multicollinearity between predictors was ignorable and all the predictors were included for further analysis. In the univariate binary logistic regression analyses, when death due to COVID-19 was regarded as the endpoint, age, number of teeth, smoking, DM, COPD, CVD, CKD, HT, and HCL had p-values of ≤0.15 (Table 3) and were included in the subsequent multivariate binary logistic regression analysis. In the multivariate analysis with backward selection, age, number of teeth, CKD, and HT remained in the final model with p-values of ≤0.15 (Table 4). When critical outcome due to COVID-19 was regarded as the endpoint, sex, age, number of teeth, smoking, DM, COPD, CVD, CKD, HT, and HCL had p-values of ≤0.15 in the univariate analyses and were included in the subsequent multivariate analysis (Table 3). In the multivariate analysis with backward selection, age, number of teeth, DM, COPD, and HT remained in the final model with p-values of ≤0.15 (Table 4).

Table 3. Univariate binary logistic regression analysis of the potential predictors for death and critical outcomes of COVID-19 based on the derivation cohort (N=285)

Potential predictors	Death (n=48, 17%)			Critical outcome (n=62, 22%)		
	B (SE)	OR (95%CI)	P	B (SE)	OR (95%CI)	P
Sex						
Female	Ref.			Ref.		
Male	0.431 (0.322)	1.539 (0.818; 2.896)	0.18	0.591 (0.295)	1.806 (1.013; 3.219)	0.05
Age	0.100 (0.016)	1.105 (1.070; 1.141)	<0.01	0.066 (0.012)	1.068 (1.043; 1.094)	<0.01
Number of teeth						
20-28 teeth	Ref.			Ref.		
1-19 teeth	2.062 (0.568)	7.865 (2.575; 24.022)	<0.01	1.497 (0.457)	4.469 (1.822; 10.962)	<0.01
0 teeth	2.160 (0.504)	8.673 (3.224; 23.337)	<0.01	1.616 (0.385)	5.030 (2.365; 10.700)	<0.01
Presence of implants						
No	Ref.			Ref.		
Yes	-0.351 (0.458)	0.704 (0.286; 1.731)	0.44	-0.299 (0.404)	0.741 (0.335; 1.640)	0.46
BMI	0.020 (0.033)	1.020 (0.956; 1.089)	0.55	0.040 (0.030)	1.041 (0.982; 1.103)	0.18
Smoking						
Non-smokers	Ref.			Ref.		
Previous smokers	0.903 (0.416)	2.466 (1.087; 5.594)	0.03	0.530 (0.359)	1.699 (0.839; 3.438)	0.14
Present smokers	0.467 (0.734)	1.596 (0.376; 6.764)	0.53	0.226 (0.643)	1.254 (0.354; 4.436)	0.73
DM						
No	Ref.			Ref.		
Yes	1.293 (0.358)	3.644 (1.806; 7.351)	<0.01	1.427 (0.338)	4.167 (2.150; 8.077)	<0.01
COPD						
No	Ref.			Ref.		
Yes	0.838 (0.400)	2.312 (1.056; 5.061)	0.04	0.883 (0.373)	2.418 (1.165; 5.021)	0.02
CVD						
No	Ref.			Ref.		
Yes	1.087 (0.351)	2.964 (1.490; 5.896)	<0.01	0.864 (0.331)	2.373 (1.240; 4.544)	<0.01
OSAS						
No	Ref.			Ref.		
Yes	0.355 (0.415)	1.427 (0.633; 3.216)	0.39	-0.023 (0.407)	0.978 (0.440; 2.170)	0.96
CKD						
No	Ref.			Ref.		
Yes	1.228 (0.456)	3.415 (1.397; 8.348)	<0.01	1.248 (0.438)	3.484 (1.475; 8.228)	<0.01
HT						
No	Ref.			Ref.		
Yes	1.762 (0.344)	5.822 (2.968; 11.419)	<0.01	1.643 (0.306)	5.172 (2.838; 9.426)	<0.01
HCL						
No	Ref.			Ref.		
Yes	0.918 (0.437)	2.504 (1.062; 5.902)	0.04	1.068 (0.409)	2.908 (1.305; 6.479)	<0.01

BMI, body mass index; DM, diabetes mellitus; COPD, chronic obstructive pulmonary diseases; CVD, cardiovascular diseases; OSA, obstructive sleep apnea; CKD, chronic kidney diseases; HT, hypertension; HCL, hypercholesterolemia; SE, standard error; OR, odds ratio; CI, confidence interval; critical outcome, death or ICU admission

Table 4. Multivariate binary logistic regression analysis with backward selection of the potential predictors for death and critical outcomes of COVID-19 based on the derivation cohort (n=285)

Potential predictors	Death due to COVID-19				Critical outcomes of COVID-19			
	B (SE)	Shrunken B	OR (95%CI)	P	B (SE)	Shrunken B	OR (95%CI)	P
Intercept	-8.430 (1.388)	-7.677		<0.01	-5.151 (0.942)	-4.661		<0.01
Age	0.080 (0.019)	0.072	1.083 (1.042; 1.125)	<0.01	0.042 (0.015)	0.037	1.043 (1.013; 1.074)	<0.01
Number of teeth								
20-28 teeth	Ref.				Ref.			
1-19 teeth	1.395 (0.642)	1.254	4.037 (1.134; 14.373)	0.03	0.839 (0.523)	0.741	2.314 (0.824; 6.503)	0.11
0 teeth	1.024 (0.581)	0.920	2.783 (0.883; 8.775)	0.08	0.756 (0.465)	0.667	2.130 (0.850; 5.339)	0.11
CKD								
No	Ref.							
Yes	0.878 (0.539)	0.789	2.406 (0.832; 6.956)	0.10				
HT								
No	Ref.				Ref.			
Yes	1.021 (0.390)	0.918	2.777 (1.288; 5.987)	<0.01	0.889 (0.359)	0.785	2.433 (1.201; 4.927)	0.01
DM								
No					Ref.			
Yes					0.652 (0.397)	0.575	1.918 (0.879; 4.188)	0.10
COPD								
No					Ref.			
Yes					0.673 (0.430)	0.594	1.959 (0.840; 4.571)	0.12

DM, diabetes mellitus; COPD, chronic obstructive pulmonary diseases; CKD, chronic kidney diseases; HT, hypertension; SE, standard error; OR, odds ratio; CI, confidence interval; critical outcome, death or ICU admission

The shrinkage factors of the models for death and critical outcome were 0.90 and 0.88, respectively. The original AUCs of the models were 0.86 (95% confidence interval [95%CI], 0.80-0.91) and 0.81 (95%CI, 0.75-0.86), respectively (Figure 2A-2B). The shrunken AUCs of the models based on the bootstrapping were 0.85 and 0.79, respectively, which indicated that the discrimination of the two models was both good to excellent. The calibration plots (Figure 3A-3B) showed that there was a good fit between the predicted probability and actual probability of the outcomes in both models because most plotted dots were lying close to the diagonal lines. The O:E ratios of the two models were 0.98 (95%CI, 0.76-1.25) and 1.00 (95%CI, 0.80-1.24), respectively, which indicated that the overall calibration of the two models was excellent. With resulting values for the HL tests of 0.64 and 0.61, the two models were shown to have good fit.

Figure 2. Discrimination ability of the predictionmodels

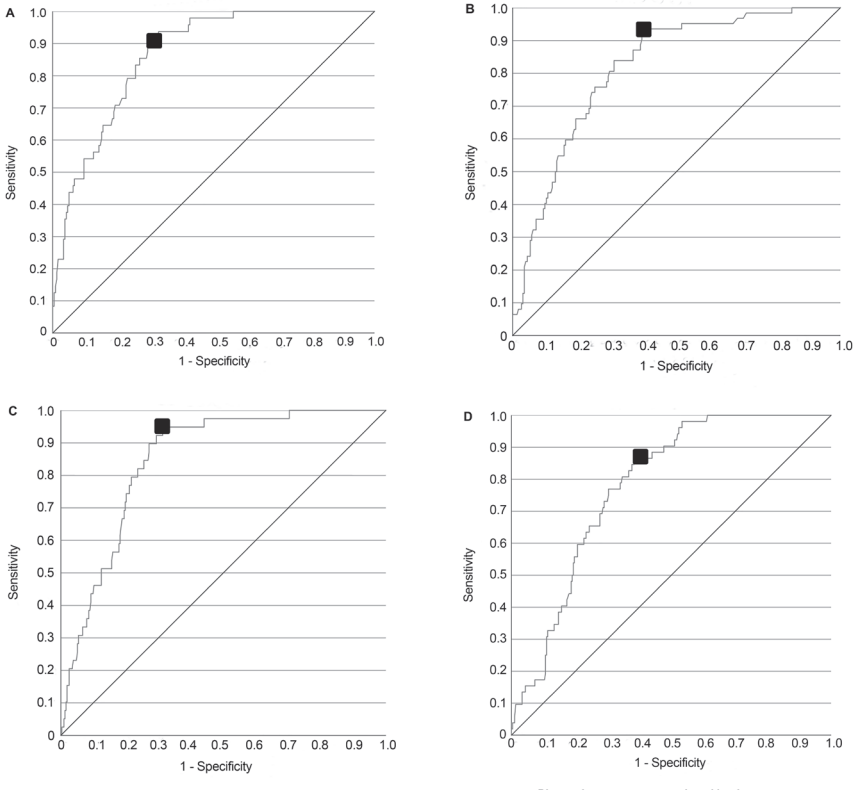

Figure 2. Discrimination ability of the prediction models for death and critical outcomes in patients with COVID-19 in derivation cohort (Panel A and B) and in validation cohort (Panel C and D). Panel A represents the ROC areas for death in the derivation cohort with a shrunken AUC of 0.85. Panel B represents the ROC areas for critical outcomes in the derivation cohort with a shrunken AUC of 0.79. Panel C represents the ROC areas for death in the validation cohort with an AUC of 0.85 (95%CI, 0.80-0.90). Panel D represents the ROC areas for critical outcomes in the validation cohort with an AUC of 0.78 (95%CI, 0.73-0.83). The diagonals represent that the models have no discrimination with an AUC of 0.50. ■ represents the optimal cutoff for the predicted probability where the sum of sensitivity and specificity is the maximum.

Figure 3. Calibration plots

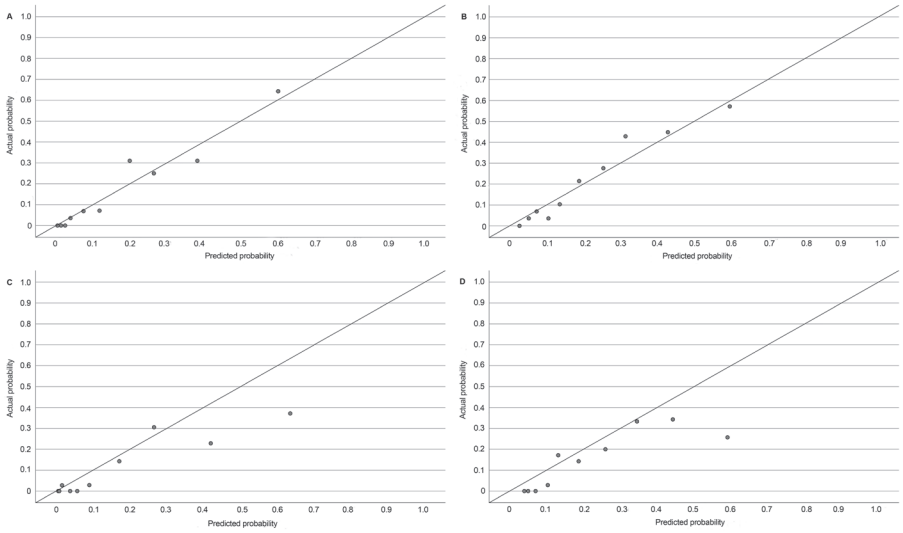

Figure 3. Calibration plots of the prediction models for death and critical outcomes in patients with COVID-19 in derivation cohort (Panels A and B) and in validation cohort (Panels C and D). Panel A represents the calibration plot for death in the derivation cohort with an O:E ratio of 0.98 (95%CI, 0.76-1.25). Panel B represents the calibration plot for critical outcomes in the derivation cohort with an O:E of 1.00 (95%CI, 0.80-1.24). Panel C represents the calibration plot for death in the validation cohort with an O:E ratio of 0.65 (95%CI, 0.49-0.85). Panel D represents the calibration plot for critical outcomes in the validation cohort with an O:E ratio of 0.67 (95%CI, 0.52-0.84). The diagonal is what would result if the predicted probability of the model was the same as the actual probability of the model so that the prediction is neither underestimated nor overestimated.

The optimal cutoffs for the predicted probability of the two models were both 0.15. Table 5 presents the prevalence, sensitivity, specificity, PPV, and NPV of the two models. When death was the endpoint, the added value of the model for ruling in the risk of death was 0.21 (95%CI, 0.11-0.31) in addition to the prevalence, while that for ruling out the risk of death was 0.14 (95%CI, 0.10-0.19) in addition to the complement of the prevalence. When critical outcome was the endpoint, the added value of the model for ruling in the risk of critical outcome was 0.18 (95%CI, 0.09-0.27) in addition to the prevalence, while that for ruling out the risk of critical outcome was 0.19 (95%CI, 0.13-0.24) in addition to the complement of the prevalence.

Table 5. Clinical values of the prediction models in derivation and validation cohort

Cohorts	Models	Prevalence (95% CI)	PPV (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Added value for ruling in the risk of the outcome events (95% CI)	Added value for ruling out the risk of the outcome events (95% CI)
Derivation cohort	For death[a]	0.17 (0.13; 0.22)	0.38 (0.29; 0.47)	0.98 (0.94; 0.99)	0.92 (0.81; 0.97)	0.70 (0.64; 0.75)	0.21 (0.11; 0.31)	0.14 (0.10; 0.19)
	For critical outcome[a]	0.22 (0.17; 0.27)	0.40 (0.32; 0.48)	0.97 (0.93; 0.99)	0.94 (0.85; 0.98)	0.61 (0.54; 0.67)	0.18 (0.09; 0.27)	0.19 (0.13; 0.24)
Validation cohort	For death[b]	0.11 (0.08; 0.15)	0.27 (0.20; 0.35)	0.99 (0.97; 1.00)	0.95 (0.84; 0.99)	0.69 (0.63; 0.74)	0.16 (0.08; 0.25)	0.10 (0.07; 0.14)
	For critical outcome[c]	0.15 (0.11; 0.19)	0.28 (0.22; 0.35)	0.96 (0.93; 0.98)	0.87 (0.75; 0.94)	0.62 (0.56; 0.67)	0.13 (0.05; 0.21)	0.11 (0.07; 0.16)

[a], the optimal cutoff for the predicted probability was 0.15; [b], the optimal cutoff for the predicted probability was 0.14; [c], the optimal cutoff for the predicted probability was 0.18; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; critical outcome, death or ICU admission

To enhance the clinical usefulness of the models, we transformed the models into a score chart (Table 6) and two linecharts (Figure 4). A clinician can easily calculate the sum scores of a patient for prediction of death and critical outcome and determine the corresponding predicted probabilities based on the sum scores by using Figure 4. The cutoffs of the sum scores of the two models were 83 and 79, respectively.

For example, a patient was diagnosed with COVID-19 in the hospital. He was 60 years old with a total of 15 remaining teeth. He had a history of COPD and HT but had no DM and CKD. Therefore, based on the score chart (Table 6), the sum score for death can be calculated as 1*60+17+0+13=90, whereas the sum score for critical outcome is 1*60+20+21+0+16=117. Both of the two sum scores of the patient were above the cutoff scores (83 and 79), and therefore the patient has a high risk of death or critical outcome due to COVID-19. Based on Figure 4, the predicted probability of the patient for death and critical outcome is around 23% and 42%.

Table 6. Score chart of the models for prediction of death and critical outcomes of COVID-19

Predictors		**Death**	**Critical outcome**
		Score	**Score**
Age		1*Age	1*Age
Number of teeth			
20-28 teeth		0	0
1-19 teeth		17	20
0 teeth		13	18
CKD	No	0	
	Yes	11	
HT	No	0	0
	Yes	13	21
DM	No		0
	Yes		16
COPD	No		0
	Yes		16
Sum score			

CKD, chronic kidney diseases; HT, hypertension; DM, diabetes mellitus; COPD, chronic obstructive pulmonary diseases; critical outcome, death or ICU admission

The algorithms for the calculation of an individual`s sum scores for death and critical outcome due to COVID-19 were presented below:

*Sum score for death = 1*Age + 17*presence of 1-19 teeth + 13* presence of 0 teeth + 11*presence of CKD + 13*presence of HT*

Sum score for critical outcome = 1*Age + *20* presence of 1-19 teeth + 18* presence of 0 teeth + 21*presence of HT + 16*presence of DM + 16*presence of COPD*

Figure 4. Line charts of the prediction models for death and critical outcomes

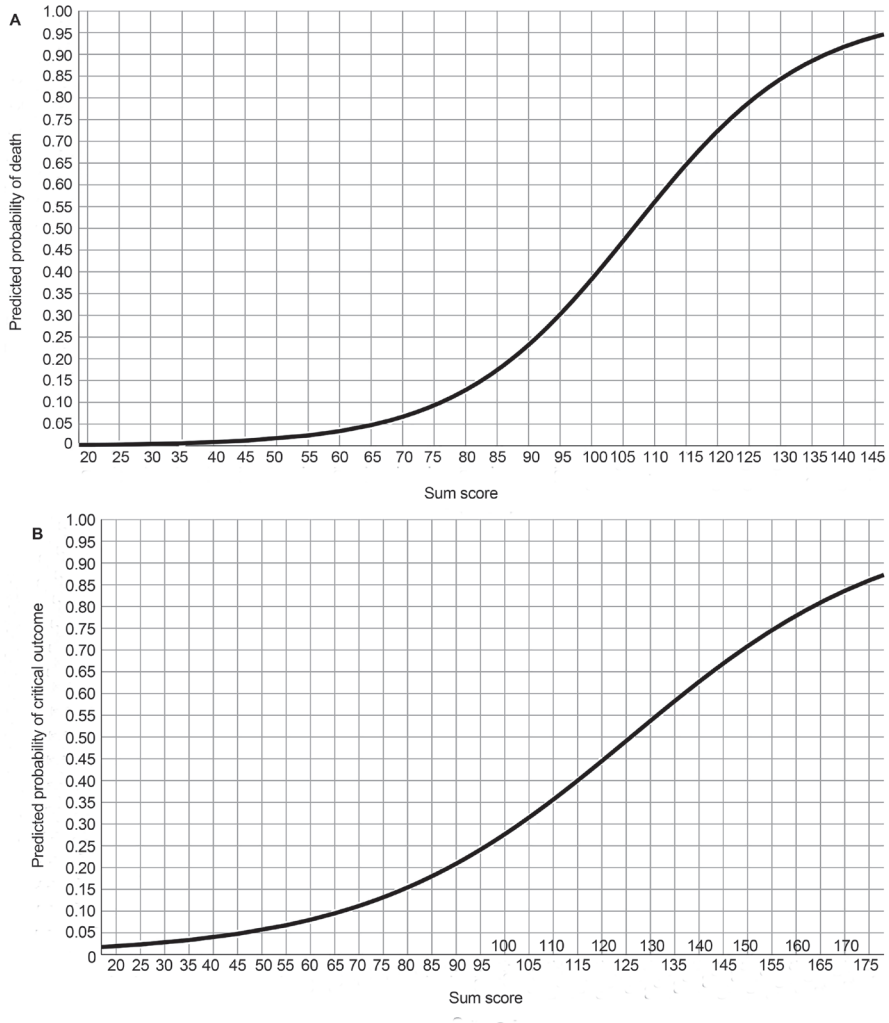

Figure 4. Line charts of the prediction models for death (Panel A) and critical outcomes (Panel B). From the line charts, the exact predicted probability of the outcome event (Axis Y) for an individual can be determined based on the sum scores (Axis X) and the curves.

External validation

The AUCs of the models for death and critical outcome based on the validation cohort were 0.85 (95%CI, 0.80-0.90) and 0.78 (95%CI, 0.73-0.83), respectively, which indicated that the discrimination of the models was good to excellent in the validation cohort (Figure 2C-2D). Based on the calibration plots (Figure 3C-3D), the calibration of the models was acceptable in general but an overestimation was observed for the patients with high predicted risks of the outcomes in both models. The overall O:E ratios of the two models were 0.65 (95%CI, 0.49-0.85) and 0.67 (95%CI, 0.52-0.84), respectively, which also indicated an overestimation of the models. The optimal cutoffs for the predicted probability of the two models were 0.14 and 0.18, respectively. Table 5 also presents the prevalence, sensitivity, specificity, PPV, and NPV of the two models based on the validation cohort. When death was the endpoint, the added value of the model for ruling in the risk of death was 0.16 (95%CI, 0.08-0.25) in addition to the prevalence, while that for ruling out the risk of death was 0.10 (95%CI, 0.07-0.14) in addition to the complement of the prevalence. When critical outcome was the endpoint, the added value of the model for ruling in the risk of critical outcome was 0.13 (95%CI, 0.05-0.21) in addition to the prevalence, while that for ruling out the risk of critical outcome was 0.11 (95%CI, 0.07-0.16) in addition to the complement of the prevalence.

Discussion

In the present study, two prediction models for death and critical outcome (death or ICU admission) in unvaccinated COVID-19 patients were derived and externally validated, based on the predictors which can be easily obtained in clinical practice, including patients` demographic characteristics, medical conditions, and dental status. In the models, older age, lower number of remaining natural teeth, and presence of CKD, HT, DM, and COPD were the important predictors for death and/or critical outcomes due to COVID-19. At present, there are already existing models for prediction of critical outcomes of COVID-19 based on various predictors on demographic characteristics, symptoms at diagnosis, medical conditions, vital signs, and laboratory or radiographic indicators. For example, Paranjape et al.[32] developed and externally validated a prediction model for critical outcomes in COVID-19 patients requiring hospitalization using DM, coronary artery disease, CKD, serum C-reactive protein, and serum lactate dehydrogenase and showed that the AUCs were 0.75 and 0.77 in the derivation and validation cohorts, respectively. Martínez-Lacalzada et al. [33] developed and externally validated a prediction model for critical outcomes on initial diagnosis of COVID-19 patients using age, dependency for activities of daily living, CVD, CKD, dyspnea, tachypnoea, confusion, systolic blood pressure, and oxygen saturation (SpO_2) or oxygen requirement, and showed that the performance of the model in both the derivation and validation cohort was good, with the AUCs of 0.82 and 0.79, respectively. Mei et al. [18] developed and externally validated a prediction model for all-cause mortality within 60 days after the diagnosis of COVID-19; they discovered age, respiratory failure, total white cell count, lymphocyte and platelet counts, plasma D-dimer and lactate dehydrogenase levels in the full version and used age, respiratory failure, coronary heart disease, renal failure, and heart failure only in a more elementary version. The full and elementary models both showed good discrimination in both derivation and validation cohorts, with AUCs ranging from 0.88 to 0.96.[18] Based on those previous prediction models, age and medical conditions were always considered important predictors, which is consistent with the findings of the present study. However, many of the previous prediction models also included predictors which were collected from the lab or radiographic tests, or clinical examinations, which may hinder the rapid and early stratification of the critical outcomes in COVID-19 patients in clinical practice. In addition, the calibration of the model was not assessed and external validation was not performed in many previous prediction models. No previous prediction models have considered dental status as the potential predictors for critical outcomes of COVID-19, while numerous studies have shown that dental status is closely associated with general health [21, 34] and prognosis of COVID-19.[11, 14, 15] Therefore, in the present study, we developed and externally validated the prediction models for critical outcomes of COVID-19 based on demographic characteristics, medical conditions, and dental status.

In the present study, number of remaining natural teeth and number of implants were included as the common clinical indicators for dental status, including dental caries and periodontal diseases. This is because the diagnosis of periodontal diseases and dental caries

requires professional dental clinical and radiographic examinations by dentists. The number of teeth and number of implants can be measured easily and reliably by clinicians or even patients themselves [35], which may facilitate the use of the prediction models in clinical practice. In the study, the number of remaining natural teeth was considered an important independent predictor for critical outcomes, in particular, for death. Tooth loss is the ultimate event representing dental pathology. Most tooth loss before middle age is caused by dental caries and in older ages, it is generally the final stage of periodontitis.[36] Dental caries is a disease with a multifactorial etiology; consumption of dietary carbohydrates is one of the most important etiological factors. High carbohydrate intake is also associated with a chronically high glycemic load that can lead to negative metabolic consequences and increased mortality risk.[37, 38] Carbohydrate intake can be a link between tooth loss and a higher mortality rate. Furthermore, tooth loss is the final sequela of periodontitis. This prolonged state of chronic inflammation with increased levels of C-Reactive Protein (CRP) is a proven risk factor for non-communicable diseases (NCDs), which are also associated with unfavorable outcomes of COVID-19.[39] Above and beyond, tooth loss might affect dietary intake and cause a malnutrition status, with negative effects for the COVID-19 progression and outcome.[40] In addition, the socio-economic position (SEP) influences lifestyle habits including smoking, obesity, physical activity, educational inequalities, and oral hygiene. Socio-economically disadvantaged individuals are more susceptible to tooth decay and periodontal disease than non-vulnerable people.[41] These socio-economic factors also play an important role in COVID-19 prevalence and mortality.[42] Therefore, SEP may accentuate the link between number of teeth and COVID-19 progression.

With regard to the performance of the two prediction models, the discrimination in both derivation and validation cohorts was found to be good to excellent, with AUCs ranging from 0.79 to 0.86. This indicated that the prediction models may have an excellent ability to differentiate the patients with the critical outcomes from those without. The calibration of the models in the derivation cohort was good based on both the calibration plots and O:E ratios. However, in the validation cohort, the O:E ratios of the prediction models were 0.65 and 0.67, respectively, which indicated an overestimation of prediction models. Based on the calibration plots, the overestimation may mainly occur when the predicted risk was larger than 0.4. This indicated that when the predicted risk of a patient was larger than 0.4, the actual risk of the patient may be lower. However, the optimal cut-off predicted risks of the models in the validation cohort were 0.14 and 0.18, respectively, which was much lower than 0.4. Therefore, even if the overestimation was present for the high predicted risks, it is not very likely to bias the risk stratification of the patients based on the optimal cut-offs.

The clinical added predictive values of the models for ruling in and out death were 0.21 and 0.14, respectively in the derivation cohort. The added values for ruling in and out critical outcomes were 0.18 and 0.19, respectively, in the derivation cohort. All the added values were statistically significant based on their 95%CIs. This indicates that if a COVID-19 patient is

predicted to have a high risk of death or critical outcome based on the models, the posterior risk of death or clinical outcome of the patient can be significantly increased by 0.21 and 0.18, respectively compared with the prevalence of death or critical outcome in the included patients. Similarly, if a patient is predicted to have low risk of death or critical outcome based on the models, the posterior probability of survival or not developing critical outcome can be significantly increased by 0.14 and 0.19, respectively, compared with the complement of the prevalence of death or critical outcome in the included patients. In the validation cohort, the added values for both ruling in and out the outcome events tended to be lower than that in the derivation cohort, but the added values were still statistically significant. The NPVs of the models in both derivation and validation cohorts were quite high, ranging from 0.96 to 0.99. This suggested that if a patient was predicted to not develop the outcome event based on the models, the patient had 96% to 99% of chance of not developing the outcome event in reality. Therefore, the ability of the models to rule out the outcome events was excellent, with only a very low false-negative rate. However, the PPVs of the models were relatively low in both cohorts, which ranged from 0.27 to 0.40. This suggested that if a patient was predicted to be at high risk for the outcome event based on the models, the patient only had 27% to 40% of chance of developing the outcome event in reality. Therefore, the prediction models may have a relatively high false-positive rate. However, considering that the prevalence (prior probability) of the outcome events was low in the included patients, the PPVs (posterior probability) still had significant added values for the prediction. Therefore, we suggest our developed models be used as an early and simple tool for rapid triage of the new COVID-19 patients at intake in hospitals, allowing further confirmation of the prognosis for the patients with high risk based on further examinations, including clinical examination, radiographic and lab tests. Therefore, false-positive prediction is not likely to negatively affect patients` health outcomes.

The present models can provide clinicians with information on the prognosis of COVID-19 patients and help clinicians with the early identification and triage of the COVID-19 patients, thus aiding in delivering proper care, reducing the fatality rates of patients, and optimizing the use of in-hospital resources.[32, 43] The present study can also increase the awareness of the clinicians on the close link between dental health and general health of individuals. In addition, the predictors included in the models could also be collected at an early stage, outside the hospitals. With the findings of the present study, primary caregivers can identify patients who may have a higher risk for poor prognosis of COVID-19 in their practice before the patients are infected. General practitioners and dentists can create the awareness with these patients about the possible prognosis if infected with COVID-19 and they can caution the patients to take necessary precautions to prevent the infection.

In interpreting the findings of the present study, some limitations should be taken into consideration. First, the number of remaining teeth of the patients was not counted at the diagnosis of COVID-19. Instead, it was counted based on the OPG taken within the past five years, and the time between the OPG and the diagnosis of COVID-19 varied between patients.

As the number of teeth may be decreasing slowly over time due to caries, periodontal diseases, trauma, etc., it can be expected that patients who took the OPG more recently may tend to have lower number of teeth than the same patients if they had taken the OPG earlier. In the analysis, the different periods in the patients between the OPG and the diagnosis were not corrected for the number of teeth, which may bias the association between number of teeth and the critical outcomes. However, we categorized the number of teeth into three ordinal categories (0 teeth, 1-19 teeth, and 20-28 teeth) in the analysis, rather than including the continuous number of teeth in the models directly. This can, on one hand, minimize the bias to a large extent, and on the other hand, simplify the counting of the number of teeth by medical clinicians and prevent the impact of miscounting on the prediction to a large extent. Second, 25% to 33% of the included patients had missing values in number of teeth and number of implants. One of the reasons for the missing values was that the patients may take the OPG more than five years before the diagnosis of COVID-19, which was outdated and may not validly reflect the present dental health. Another reason was that those patients did not have an indication for an OPG. Therefore, those missing values were assumed to missing at random (MAR). The multiple imputation technique was used to deal with the missing values, which can preserve the sample size and reduce the bias of the MAR data caused by the missing values when the proportion of missingness is relatively large.[44] Third, the predictors on medical conditions were all routinely collected from patients` electronic health records, which was more accessible, informative, standardized, and reliable than patients` self-reported methods.[45, 46] This, however, may hinder the generalizability of the prediction models in the hospitals where the patients` health information was not well and comprehensively documented. Fourth, the COVID variant types were not identified in the included patients, therefore, the models were not corrected for variant types. All the included patients were diagnosed as COVID-19 between January 2020 and July 2021, which indicated that those patients probably had a mixture of variant types, including Alpha, Beta, Gamma, and Delta.[47] Those variant types may have different clinical manifestations, transmissibility, morbidity, and mortality of COVID-19.[48] Therefore, not correcting for the variate types of the COVID in the models may impair the performance of the prediction models. In addition, from December 2021, the Omicron variant rose rapidly around the world and became dominant in many countries, including the Netherlands. Omicron seems to be milder with lower mortality but more transmissible than the previous variants based on the very limited evidence so far.[49-51] Whether the prediction models can be generalized to the patients with Omicron variants is still unknown and needs to be further validated. Fifth, a smaller number of events relative to the high number of predictors is a common limitation for multivariate prediction models. The events per variable (EPV) of 10 is the widely used rule of thumb for multivariate logistic regression analyses to obtain a reliable outcome when developing the prediction models. [52, 53] As for the validation of the prediction models, it is recommended to include at least 100 events.[54] The present study, however, did not meet the criteria because of the small sample size and the low prevalence of the outcome events. To reduce the number of predictors included in the multivariate models, univariate analyses were used to pre-screen the predictors

in the study. Besides, a less stringent threshold of the significance level of 0.15 was used in modeling for the selection and exclusion of potential predictors, which may prevent the false exclusion of the important predictors to a large extent. In this way, the negative consequence caused by the small sample size could be reduced to a large extent.

Future researchers are suggested to further validate and update the prediction models in the COVID-19 patients with the Omicron variant and the vaccinated patients. Besides, it is recommended to assess whether the performance of the models improves when adding the variant types of COVID-19 as a separate predictor.

In conclusion, age, number of remaining natural teeth, CKD, HT, DM, and COPD were the important predictors for death and/or critical outcomes due to COVID-19 in unvaccinated COVID-19 patients in the hospital setting. The performance of the models, in aspects of discrimination and calibration, was acceptable in both derivation and validation cohorts. The added predictive values were considerable for both ruling in and ruling out the death and the critical outcome in decision-making. The models can be used as a reliable screening tool for early and rapid risk stratification of unvaccinated COVID-19 patients at intake in hospital setting.

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PART III

Dental status as a window to general health

CHAPTER 8

General discussion, clinical implications & future perspectives

GENERAL DISCUSSION

The link between dental status and general health is fascinating and important. Most research in this field focused on the association between periodontitis and cardiovascular diseases as well as diabetes mellitus. However, a growing body of literature suggests that there is a link between periodontitis and other systemic diseases, including rheumatoid arthritis, certain cancers, respiratory diseases, cognitive disorders and adverse pregnancy outcomes. [1], [2] Moreover, analyses of trial registers showed that even fifty-seven systemic conditions are hypothesized to be linked with periodontitis.[3] Attention for more associated systemic diseases is not the only topic that has changed in this research field. During the past years, other dental conditions, next to periodontitis, have gained ground for linking dental status to general health. Ultimately, tooth loss, the final event representing dental pathology, is associated with multiple systemic comorbidities and higher risks for all-cause mortality.[4], [5]

Most previous literature on this topic has been initiated to find causality between dental pathologies and systemic diseases. But to this day, unidirectional and bidirectional causality remains an ongoing topic of debate.[6], [7] The work presented in this thesis, did not find causality, but added further knowledge to the once-wide gap between dentistry and general medicine, elaborated in two essential general health conditions: cardiovascular diseases and COVID-19. The genetic-, immune-, and risk- profile of an individual are designated as common denominators. Subsequently, a more widespread and general statement on the importance of the dental status as a window to general health is made.

Genetic profile

Genetic factors associated with the susceptibility, severity and the development of periodontitis and dental caries have pointed to a number of gene variants of interest.[8] Additional insight has been found in studies in which periodontitis is analyzed in combination with other conditions, such as cardiovascular diseases. It has been hypothesized that, since periodontitis and cardiovascular disease are both characterized by disproportional inflammatory response, they may share genetic background.[9] Combined genetic and functional studies point to immunogenetic blueprints in which immune fitness is disturbed. This has conducted to the suggestion of “a signature” of more than 65 genes, involving inflammatory features and association with other health conditions, especially cardiovascular diseases.[10]

Immune profile

The susceptibility of a host is a fundamental link to develop systemic diseases. Currently, insufficient immune fitness has prompted to be at the base of host susceptibility to many systemic diseases. Immune fitness refers to the way individuals deal with the challenges and disturbances encountered during life, including inflammation resolving mechanisms.

Correspondingly, a resilient immune system is capable of returning to homeostasis after an external challenge. It will fight infections and it will down-regulate the immune response once an infection is cleared to prevent harmful responses against the tissues of the own body. Immune fitness changes during life and is determined by genetic, environmental and local factors. Lack of sufficient immune fitness is acknowledged to play an important role in the co-occurrences of dental pathology and systemic diseases.[10], [11]

Risk profile

Low socio-economic position (SEP) is associated with poor lifestyle habits including smoking, obesity, physical activity, harmful use of alcohol, and poor oral hygiene.[12] Socio-economically disadvantaged individuals are more susceptible to tooth decay and periodontal disease than non-vulnerable people.[13] These socio-economic factors and the related shared risk factors of dental pathologies and some systemic conditions, also have a significant and consistent impact on the global burden of diseases and their mortality and morbidity.[14] These shared risk factors should always be taken into account when discussing the link between poor dental health and several systemic diseases.

Dental status a window to general health

Dental pathologies and various systemic diseases are based on overlapping complex pathophysiological mechanisms, with genetic and epigenetic factors that interact with lifestyle and environmental factors. The resulting “susceptibility” of the host seems the fundament of the link between dental status and general health. Evidence for possible causal relations is still lacking, and the urgency for causal explanations is increasingly moving to the background.

CLINICAL IMPLICATIONS & FUTURE PERSPECTIVES

Teeth are an integral part of the body, supporting essential physical and psychological functions. The dental status reflects the capability to adapt to physiological changes throughout life and could be used as an indicator for morbidity and mortality of systemic diseases. Nevertheless, dental status is still a neglected issue. That's exactly why higher awareness about how dental status could mirror the general health status should be added to the global health agenda. Simplifying the dental status to "tooth loss", as the ultimate sequela of dental pathology and poor oral health, makes it a quick and easily accessible marker for general health. Consequently, inspection of the dentition must be adapted as a standard widespread diagnostic tool by all health workers.

In conclusion, the work presented in this thesis added further knowledge to the once-wide gap between dentistry and general medicine; screening the dental status of patients may help to identify the person at risk for morbidity and mortality of systemic diseases.

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CHAPTER 9

Summary (English)

SUMMARY (English)

The link between dental status and general health is fascinating and relevant. Most precedent literature in this field tries to find causality between dental pathology and systemic diseases, mainly based on derivative parameters. This thesis provides new insights into this link elaborated in two essential general health conditions: cardiovascular diseases (as the leading cause of global mortality) **(Part I)** and COVID-19 (as a recent example of a worldwide pandemic) **(Part II)**. Consequently, a more widespread and general statement on this exciting and above all important topic is discussed in **Part III**.

Chapter 1 provides a general introduction to the background and significance of the link between dental status and general health.

Part I – Dental status as a window to cardiovascular disease

Most research in this field regards the association between periodontitis and cardiovascular disease. **Chapter 2** presents a review summarizing the literature on this topic. Although causal mechanisms are still lacking, several pathophysiological and epidemiological pathways have been suggested. Pathophysiological pathways involve a *direct mechanism:* invasion of periodontal pathogens into atherosclerotic plaques and *indirect mechanisms:* increased level of systemic inflammation, increased platelet activation and molecular mimicry. Moreover, the link may also be explained by a shared genetic basis of periodontitis and cardiovascular disease, that has recently been demonstrated.

The described previous studies predominantly used surrogate biomarkers to investigate the association between cardiovascular disease and periodontitis, and to evaluate the effects of periodontal treatment. **Chapter 3-5** describes studies based on more direct parameters.

Chapter 3 explores the association between Coronary Artery Calcium (CAC) scores, investigated by a cardiac CT-scan, and dental pathology determined on a dental panoramic radiograph. The results of this retrospective study show a statistically significant association between the number of missing teeth and the CAC score. However, after multivariate correction for age, sex, and other well-known risk factors for cardiovascular disease, the significant correlation was no longer present. Furthermore, the results show a tendency for more teeth with peri-apical lesions and a higher percentage of mean alveolar bone loss in the group with the highest CAC scores.

Chapter 4 describes the results of a cross-sectional study that aimed to investigate the association between the inflammatory burden of periodontitis and the presence and extent of coronary calcification. Full-mouth examinations were performed by a periodontist to determine the Periodontal Inflamed Surface Area (PISA) score and other dental parameters. To

evaluate the cardiovascular conditions, CAC scores, endothelial function assessments by the EndoPAT™, and several physical and biochemical examinations were performed. Seventy-one subjects (41 periodontitis patients and 30 controls) were included. Elevated CAC scores and endothelial dysfunction were not significantly related to PISA score or dental health. Although the PISA score was significantly related to the Framingham and Reynolds CVD risk predictors, after correction for confounders this was no longer significant. The same applied to the significant associations between tooth loss, dental plaque and bleeding scores and the CVD risk predictors. **Chapter 5** shows a follow -up of the patients included in the study described in chapter 4. One year after completing periodontal (non-surgical and surgical) treatment of the periodontitis patients and one year after inclusion of the controls, the full mouth examination and measurements of endothelial function and other physical and biochemical cardiovascular parameters were repeated. This study did not find an improvement of endothelial function or other cardiovascular parameters after highly effective periodontal treatment.

Part II – Dental status as a window to COVID-19

Chapter 6 introduces the exploration of an association between dental status and severity of COVID-19. In this retrospective, cohort study we observed a statistically significant relation between the COVID-19 severity with alveolar bone loss and tooth loss. However, when adjusted for the well-known risk factors of COVID-19, these dental parameters were not identified as independent risk factors for the course and outcome of COVID-19 in our study population. The results of this study contributed to the design of the next study, described in **Chapter 7.** This study aimed to develop and externally validate prediction models for critical outcomes of COVID-19 based on demographic characteristics, medical conditions, and dental status. Interestingly, it was found that beside age, and several medical conditions, the number of remaining natural teeth, proves to be an important predictor for death and/or critical outcome due to COVID-19.

Part III - Dental status a window to general health

Dental pathologies and various systemic diseases are based on overlapping complex pathophysiological mechanisms, with genetic and epigenetic factors that interact with lifestyle and environmental factors. The resulting “susceptibility” of the host seems the fundament of the link between dental status and general health. Evidence for possible causal relations is still lacking, and the urgency for causal explanations is increasingly moving to the background.

Dental status is still a neglected issue. That’s exactly why higher awareness about how dental status could mirror the general health status should be added to the global health agenda.

Simplifying the dental status to “tooth loss”, as the ultimate sequela of dental pathology and poor oral health, makes it a quick and easily accessible marker for general health. Consequently,

inspection of the dentition must be adapted as a standard widespread diagnostic tool by all health workers.

In conclusion, the work presented in this thesis added further knowledge to the once-wide gap between dentistry and general medicine; screening the dental status of patients may help to identify the person at risk for morbidity and mortality of systemic diseases.

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CHAPTER 10

Samenvatting (Nederlands)

SAMENVATTING (Nederlands)

Het raakvlak tussen de conditie van het gebit en algemene gezondheid is buitengewoon interessant en relevant. Het meeste onderzoek op dit gebied is op zoek naar een causaal verband tussen dentale pathologie en systemische ziekten, voornamelijk gebaseerd op afgeleide parameters. Dit proefschrift biedt nieuwe inzichten in deze interactie, uitgewerkt in twee essentiële algemene gezondheidsproblemen: hart- en vaatziekten (als de belangrijkste oorzaak van wereldwijde sterfte) **(Deel I)** en COVID-19 (als recent voorbeeld van een wereldwijde pandemie) **(Deel II).** Hiermee wordt een globaler en meer wijdverbreid statement gemaakt over dit boeiende en vooral belangrijke onderwerp (**Deel III)**.

Hoofdstuk 1 geeft een algemene inleiding over de achtergrond en het belang van het verband tussen de conditie van het gebit en de algemene gezondheid.

Deel I – De conditie van het gebit als venster voor hart- en vaatziekten

De meeste onderzoeken op dit gebied gaan over het verband tussen parodontitis en hart- en vaatziekten (HVZ). **Hoofdstuk 2** geeft een overzicht van de literatuur over dit onderwerp. Hoewel eenduidig bewijs voor causale mechanismen nog steeds ontbreken, worden er verschillende pathofysiologische en epidemiologische routes gesuggereerd. Pathofysiologische routes omvatten een *direct mechanisme*: invasie van parodontale pathogenen in atherosclerotische plaques en *indirecte mechanismen*: verhoogd niveau van systemische ontsteking, verhoogde bloedplaatjes activatie en moleculaire mimiek. Bovendien kan het verband ook worden verklaard door een gedeelde genetische basis van parodontitis en hart- en vaatziekten, die recentelijk is aangetoond.

De beschreven eerdere studies gebruikten voornamelijk afgeleide biomarkers om het verband tussen hart- en vaatziekten en parodontitis te onderzoeken en om de mogelijke effecten van parodontale behandeling te evalueren. **Hoofdstuk 3-5** beschrijven studies gebaseerd op meer directe parameters.

In **Hoofdstuk 3** werd de associatie onderzocht tussen coronaire calcium (CAC) scores, vastgesteld door een CT-scan van het hart, en dentale conditie, bepaald op een panoramische röntgenfoto. De resultaten van deze retrospectieve studie laten een statistisch significante relatie zien tussen het aantal ontbrekende gebitselementen en de CAC-score. Na multivariate correctie voor leeftijd, geslacht en andere bekende risicofactoren voor HVZ was het significante verband echter niet meer aanwezig. Verder toonden de resultaten een trend waarbij in de groep patiënten met de hoogste CAC scores, meer gebitselementen met peri-apicale laesies en een hoger percentage van alveolair botverlies werd waargenomen.

Hoofdstuk 4 beschrijft de resultaten van een cross-sectionele studie die als doel had de associatie tussen de inflammatoire belasting van parodontitis en de aanwezigheid en mate van coronaire calcificatie te onderzoeken. Hierbij werd een volledig mondonderzoek uitgevoerd door een parodontoloog om de ontstekingslast veroorzaakt door parodontitis te kwalificeren (middels de Periodontal Inflamed Surface Area (PISA)-score) en om andere dentale parameters te bepalen. Om de cardiovasculaire conditie te beoordelen, werden de CAC score en endotheel functie (middels EndoPAT™) bepaald. Daarnaast werden verschillende lichamelijke en biochemische onderzoeken uitgevoerd. Eenenzeventig proefpersonen (41 parodontitis patiënten en 30 controles) werden geïncludeerd. Verhoogde CAC-scores en endotheel disfunctie waren niet significant gerelateerd aan de PISA score of dentale conditie. Hoewel de PISA-score significant gerelateerd was aan de Framingham en Reynolds cardiovasculaire risicovoorspellers, was dit na correctie voor confounders niet langer significant. Hetzelfde gold voor de significante associaties tussen tandverlies, tandplak en bloedingsscores en de cardiovasculaire risicovoorspellers. **Hoofdstuk 5** toont de follow-up van de patiënten die zijn geïncludeerd in de studie beschreven in hoofdstuk 4. Een jaar na het voltooien van de parodontale (niet-chirurgische en chirurgische) behandeling van de parodontitispatiënten en een jaar na inclusie van de controlegroep, werden het mondonderzoek, de metingen van de endotheel functie en andere lichamelijke en biochemische onderzoeken herhaald. Deze studie vond geen verbetering van de endotheel functie of andere cardiovasculaire parameters na zeer effectieve parodontale behandeling.

Deel II – De conditie van het gebit als venster voor COVID-19

Hoofdstuk 6 introduceert de verkenning van een verband tussen de conditie van het gebit en de ernst van COVID-19. In deze retrospectieve cohortstudie werd een statistisch significante relatie waargenomen tussen alveolair botverlies en tandverlies en de ernst van COVID-19. Na correctie voor de bekende risicofactoren van COVID-19, werden deze dentale parameters echter niet meer gezien als onafhankelijke risicofactoren voor het beloop en de uitkomst van COVID-19. De resultaten van deze studie hebben wel bijgedragen aan het ontwerp van een voortvloeiende studie, beschreven in **hoofdstuk 7**. Deze studie was gericht op het ontwikkelen en extern valideren van voorspellingsmodellen voor kritieke uitkomsten van COVID-19 op basis van demografische kenmerken, medische aandoeningen en conditie van het gebit. Interessant genoeg bleek dat naast leeftijd en verschillende medische aandoeningen, het aantal resterende natuurlijke gebitselementen een belangrijke voorspeller is voor overlijden en/of kritieke uitkomst als gevolg van COVID-19.

Deel III De conditie van het gebit als venster voor de algemene gezondheid

Aandoeningen van het gebit en verschillende systemische ziekten zijn gebaseerd op overlappende complexe pathofysiologische mechanismen, met genetische en epigenetische factoren die interageren met levensstijl en omgevingsfactoren. De resulterende “gevoeligheid” van de gastheer lijkt het fundament van het verband tussen de conditie van het gebit en algemene gezondheid. Bewijs voor mogelijke causale verbanden ontbreekt nog steeds, en de noodzaak voor causale verklaringen verdwijnt steeds meer naar de achtergrond.

De conditie van het gebit is nog steeds van ondergeschikt belang. Dat is exact de reden waarom een groter bewustzijn over “het gebit als weerspiegeling van de algemene gezondheid”, moet worden toegevoegd aan de wereldwijde gezondheidsagenda.

Door de dentale conditie te vereenvoudigen tot “tandverlies”, als het ultieme gevolg van dentale pathologie en slechte mondgezondheid, lijkt dit een snelle en gemakkelijk toegankelijke marker voor de algemene gezondheid. Uiteindelijk moet inspectie van het gebit door alle zorgverleners worden geaccepteerd als een standaard, globaal diagnostisch hulpmiddel.

Samenvattend heeft dit proefschrift meer kennis toegevoegd aan de eens zo grote kloof tussen tandheelkunde en algemene geneeskunde; screening van de conditie van het gebit kan helpen bij het identificeren van de patiënten met een verhoogd risico op morbiditeit en mortaliteit door systemische ziekten.

APPENDICES

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Bol.com, Amazon.nl, Zalando.nl, etc, etc. Zonder jullie **premium memberships** waren er heel wat verjaardagen voorbijgegaan zonder cadeautjes, en liep ons hele gezin er een stuk minder fraai bij. Online shoppen is een grote redding voor mij geweest de afgelopen jaren!

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"Mama, wanneer ben je klaar?" ~~Bijna~~. Nu!

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ABOUT THE AUTHOR

Marie-Chris Donders was born in Arnhem on May 13th, 1985. She Spent her childhood with her parents and awesome brother and sister in Arnhem and Friesland.

After graduation in 2003, she moved to Amsterdam to study Medicine at the University of Amsterdam (UvA). During her bachelor, she was part of the AmsteRdam REsuscitation STudies (ARREST) team as a research student, supervised by dr. J. Berdowski and dr. R.W. Koster. During her study, in 2007, she worked for six months as a volunteer in the medical outreach program and hospice for Child-Support-Ghana in Wa.

Marie-Chris got fascinated by the Oral and Maxillofacial (OMF) surgery at the end of her Medical study. Therefore, after obtaining her Medical Degree in 2010, she started to study Dentistry at the Academic Centre for Dentistry Amsterdam (ACTA - UvA). Not much later, she started her PhD research project under inspiring supervision of prof. dr. J. de Lange, prof. dr. B.G. Loos and prof. dr. A.W.J. van 't Hof. In 2014 she graduated and became a resident in Oral and Maxillofacial Surgery at the Amsterdam University Medical Center (Amsterdam UMC, location AMC) in 2015 (Head of the department: Prof. dr. J. de Lange).

As a part of the OMFS training program she was seconded as a resident to the Netherlands Cancer Institute (NKI) Antoni van Leeuwenhoek (supervised by prof. dr. L.E. Smeele) and the Isala Clinics in Zwolle (supervised by dr. E.M. Baas). At the beginning of 2020 she was registered as an Oral and Maxillofacial surgeon and directly started as a consultant in the Isala Clinics in Zwolle and Treant hospital in Hoogeveen.

Marie-Chris lives together with her loving partner Jeroen Frerichs. They are proud parents of Puck (2013) and Quinten (2017).

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