Periodontitis and coronary artery disease
Studies on the association between periodontitis and coronary artery disease

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With love to Pelle, Greta and Olle

"Utan tvivel är man inte klok."
Tage Danielsson, 1928-1985
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ABSTRACT

Periodontitis and coronary artery disease (CAD) are highly prevalent in Sweden’s population; both diseases have complicated pathogeneses and clinical manifestations due to immune-system triggered inflammation. Research in recent years reported that inflammation is a significant active participant in many chronic diseases. The literature described a CAD-periodontitis association, but underlying mechanisms are not fully understood. It is important to acquire knowledge about how periodontitis might influence CAD, which is one of the major causes of illness and death in Western countries. Because periodontitis can be treated, this knowledge, when complemented with more knowledge about the CAD-periodontitis association, could lead to CAD prevention.

The overall aim of studies reported in this thesis were to investigate the CAD-periodontitis association, and specifically, to: (i) compare periodontal conditions in patients with CAD and subjects without a history of CAD; (ii) study whether or not periodontal status influences outcomes in known CAD over an 8-year period; (iii) study whether or not concentrations and biological activity of hepatocyte growth factor (HGF) in serum from patients with severe CAD are different – depending on whether or not the subjects had periodontitis; and (iv) study concentrations and biological activity of hepatocyte growth factor in serum, saliva, and gingival crevicular fluid in healthy subjects with or without periodontitis. Here is a brief summary:

In study I, 161 patients with CAD and 162 controls were compared regarding periodontal disease prevalence and severity. CAD patients had significant coronary stenosis and underwent percutaneous coronary intervention (PCI) or coronary artery by-pass grafts (CABG). Healthy controls were recruited from Sweden’s population database. Twenty-five per cent of the CAD patients had severe periodontitis, compared to 8% of the controls. In a multiple logistic regression analysis (controlled for age and smoking), severe periodontitis indicated an odds ratio of 5.74 (2.07–15.90) for CAD.

Study II: Periodontal status was re-examined in 126 CAD patients and 121 controls from the initial sample after 8 years. Periodontal status at baseline was analysed and related to CAD endpoints (i.e., myocardial infarction, new PCI or CABG or death due to CAD) recorded from patients’ medical records and from the death index maintained by the National Board of Health and Welfare. The difference in periodontitis prevalence and severity between the
Abstract
two groups remained unchanged during the 8-year follow up. No significant differences were found regarding CAD endpoints during follow-up in relation to baseline periodontal status in the CAD-patient group.

In study III, higher HGF serum concentrations (p<0.001) were found in CAD patients, compared to healthy blood donors, which reflects chronic inflammation. In CAD patients without periodontitis, HGF concentrations increased significantly 24 hours after PCI – in parallel with increased HGF biological activity. In CAD patients with periodontitis, only small fluctuations were seen in HGF values, i.e., concentration and biological activity. HGF biological activity was temporarily elevated after PCI but only in patients without periodontitis. Thus chronic inflammation related to periodontitis might reduce HGF biological activity.

In study IV, HGF concentration and biological activity in saliva, in gingival crevicular fluid (GCF), and serum were compared between 30 generally healthy subjects with severe untreated periodontitis and 30 healthy subjects without periodontitis. Compared to periodontally healthy controls, periodontal patients showed higher HGF concentrations in saliva p<0.001, gingival crevicular fluid p<0.0001, and in serum p<0.001. HGF biological activity (measured as the binding affinity to its HSPG and c-MET receptors) was significantly reduced in saliva (p<0.0001) and GCF samples (p<0.0001 for HSPG and p<0.01 for c-MET) from periodontitis patients. The only significant difference in serum samples was an increase in c-MET binding three minutes after subgingival debridement in periodontitis patients (p<0.05), which might reflect that patients had active bursts of periodontitis.

In conclusion, CAD patients more often showed severe periodontitis but there were no differences in CAD endpoints during the eight-year follow-up in relation to baseline periodontal status. Periodontitis seems to influence HGF concentration and biological activity in CAD patients, but studies on factors that cause lower HGF biological activity are necessary – to find out if periodontal treatment influences HGF biological activity. Healthy periodontitis patients had higher HGF concentrations locally and systemically, but biological activity was reduced. This might indicate that periodontitis can influence wound healing and tissue repair in other body parts.

Målet med denna avhandling var att jämföra parodontala förhållanden hos patienter med kranskärlssjukdom med friska individer, samt att studera påverkan av parodontit på kranskärlssjukdom under längre tid. Dessutom studerades koncentration och biologisk aktivitet av ett protein viktigt för sårläkning och vävnadsreparation, hepatocyte growth factor (HGF), som har föreslagits som markör för både parodontit och kranskärlssjukdom.

Avhandlingen baseras på fyra studier.

I studie I jämfördes förekomst och grad av parodontit hos 161 kranskärlssjuka patienter med 162 matchade kontrollindivider utan kranskärlssjukdom. Alla patienter med kranskärlssjukdom hade genomgått kranskärlsröntgen som visade omfattande förrådningar i kranskärlen vilka behandlades med ballongutvidgning eller by-pass operation. 25 % av patienterna hade grav parodontit jämfört med 8 % procent av kontroll individerna vilket var en statistiskt säkerställd skillnad. Studie II: Efter 8 år undersöckes ånyo 126 kranskärlssjuka patienter och 121 friska kontroll individer från den första studien. Kranskärlssjuka patienter som drabbades av nya hjärtkomplikationer (hjärtinfarkt, behov av ny ballongutvidgning eller by-pass operation eller död till följd av kranskärlssjukdom) identifierades genom patienternas journaler från hjärtkliniken samt Socialstyrelsens dödregister. Inga signifikanta korrelationer noterades mellan parodontit vid ursprungens undersökningen och incidensen av nya hjärtkomplikationer. I hela den ursprungliga gruppen på 323 individer såg vi en tendens till fler hjärthändelser hos individer som vid första undersökningen hade uppvisat grav parodontit, men denna tendens var inte statistiskt säkerställd.
Populärvetenskaplig sammanfattning

Studie III visade statistiskt säkerställd högre koncentration av HGF i serum hos 36 patienter med kranskärlssjukdom jämfört med 56 friska blodgivare. Hos kranskärlssjuka patienter utan parodontit ökade koncentrationen av HGF 24 timmar efter ballongutvidgning parallellt med att den biologiska aktiviteten av HGF ökade. Hos kranskärlssjuka patienter med parodontit kunde vi endast se mindre förändringar. Detta kan tyda på att kronisk inflammation i form av parodontit försämrar den biologiska aktiviteten av HGF.

I studie IV jämfördes koncentration av HGF i saliv, gingival vätska (från tandköttsfickor) och serum hos 30 friska individer med grav parodontit och 30 friska personer utan parodontit. Parodontitpatienterna hade statistiskt säkerställd högre koncentrationer av HGF i saliv, gingival vätska och serum. Hos patienter med parodontit hade HGF statistiskt säkerställd lägre biologisk aktivitet i saliv, i vätska från tandköttsfickor och i serum efter depuration (rengöring av tandköttsfickor).

PREFACE

The thesis reports on these original studies, which are referred to in the text by their Roman numerals.


ABBREVIATIONS AND DEFINITIONS

Abbreviations

ACS  acute coronary syndrome
A. actinomyctemcomitans  Aggregatibacter actinomyctemcomitans
AL  attachment level
AMI  acute myocardial infarction
BOP  bleeding on probing
c-MET  mesenchymal epithelial transition factor
cMTI  carotid intima media thickness
CABG  coronary artery by-pass graft
CAD  coronary artery disease
CD  cluster of differentiation
cIMA  carotid intima-media area
cIMT  carotid intima-media thickness
CVD  coronary vascular disease
HDL  high-density lipoproteins
HGF  hepatocyte growth factor
hs-CRP  high-sensitivity serum C-reactive protein
HSP  heat shock proteins
HSPG  heparan sulfate proteoglycan
ICAM-1  inter-cellular adhesion molecule-1
IL  interleukin
IMT  carotid artery intima-media thickness
LDL  low-density lipoproteins
LPS  lipopolysaccharide
MI  myocardial infarction
MHC  major histocompatibility complex
MMP  matrix metalloproteinase
MPC-1  monocyte chemoattractant protein-1
non-STEMI  non-ST segment elevation myocardial infarction
OR  odds ratio
P. gingivalis  Porphyromonas gingivalis
PCI  percutaneous coronary intervention
PCTA  percutaneous transluminal coronary angioplasty
PD  pocket depth
Abbreviations and definitions

PDGF: platelet derived growth factor
PGE2: prostaglandin E2
PPD: probing pocket depth
PMN: polymorphonuclear leukocytes
RANKL: receptor activator of nuclear factor kappa B ligand
RR: risk ratio
SPR: surface plasmon resonance
SR: scavenger receptor
SRP: scaling and root planning
STEMI: ST segment elevation myocardial infarction
TNF-α: tumour necrosis factor α
TNF-β: tumour necrosis factor β
TLR: toll-like receptor
VCAM-1: vascular endothelial adhesion molecule-1

Definitions

**Acute coronary syndrome.** Atherosclerotic plaques rupture that leads to thrombus formation in coronary arteries (coronary thrombosis). Clinical manifestations are myocardial infarction (STEMI or non-STEMI) or instable angina.

**Acute myocardial infarction.** Blood supply interruption to a heart section that causes cell death at the myocardium.

**Angina pectoris.** Chest pain due to ischemia of the heart muscle.

**Atherosclerosis.** Artery wall thickness due to plaques.

**Coronary artery bypass graft.** Surgical method used to graft a healthy artery or vein to a blocked coronary artery.

**Coronary artery disease.** Conditions in which plaques build in the coronary arteries due to atherosclerosis.

**Cardiovascular disease.** Diseases that affect the cardiovascular system, the term is usually used for diseases related to atherosclerosis.

**Ischemic heart disease.** Reduced blood supply to the heart muscle, usually due to atherosclerosis in coronary arteries. Clinical manifestations include acute myocardial infarction, stable angina, unstable angina, or sudden death.
**Abbreviations and definitions**

**Percutaneous coronary intervention.** Non-surgical widening procedure to treat narrowed coronary arteries; synonymous with percutaneous transluminal coronary angioplasty.

**Non-STEMI.** A type of heart attack in which thrombus partially blocks the coronary artery; characteristic changes of the negative T wave or ST segment depressions on electrocardiography occur.

**STEMI**
A type of heart attack in which the coronary artery is completely blocked; characteristic changes of ST segment elevations on electrocardiography (ECG) occur.
INTRODUCTION

As a dentist and periodontics consultant, I treat patients with severe periodontal disease. These patients suffer from chronic inflammatory disease that (i) leads to destruction of tooth periodontium and (ii) results in tooth loss. The pathogenesis of periodontitis is complex; it includes several risk factors such as neglected oral hygiene, tobacco and smoking habits impaired general health, and genetic susceptibility (Heitz-Mayfield et al., 2005; Persson, 2008; Stabholz et al., 2010). Consequently, patients with severe periodontitis may have one or several general diseases, including coronary artery disease (hereafter, CAD).

CAD, a consequence of inflammatory and immunological reactions in the coronary arteries, is a multi-factor disease with many underlying risk factors; several are known but not explained in all cases. CAD is one of the main reasons for premature deaths in industrial countries. In Europe, 40% of deaths are caused by cardiovascular disease, i.e., about 2 million deaths annually.¹

During the past decade, the literature described growing evidence for a CAD-periodontitis association. It is reasonable to believe that periodontitis has an influence on processes involved in CAD or might even be a risk factor. Work that led to this thesis intended to (i) contribute to this research area, (ii) focus on the epidemiological relationship between CAD and periodontitis, (iii) study the connection between the two diseases and to study one systemic inflammatory factor, Hepatocyte growth factor (HGF), involved in systemic inflammation and wound healing.

Historical perspective

Impact of oral health on general health has been discussed for centuries. In the past, there was a belief that bad teeth could promote general diseases, especially those with unknown aetiology.

¹ http://ec.europa.eu/health-eu/health_problems/cardiovascular_diseases
In 1900, William Hunter introduced the oral sepsis term. He suggested that poisoning from oral diseases, such as pyorrhea alveolaris, could be particularly detrimental to general health, especially from infectious diseases caused by pus organisms, e.g., ulcerative endocarditis (Hunter, 1900).

In 1930, Billings further developed the theory of focal infection and suggested that pathogens from one local primary focus, under appropriate circumstances, could spread via the blood and lymph fluid thus causing disease elsewhere. Rheumatic, heart, and kidney diseases were some of the possible targets (Billings, 1930). Rosenow, Billings’s research associate, injected strains of streptococci from patients with chronic diseases, e.g., arthritis, in animals and concluded that the animal tended to develop lesions (Rosenow, 1930).

Focal infection was suggested as a factor that contributed to several general diseases, especially in joints and cardiac valves (Cecil, 1934). Unfortunately, Rosenow’s recommendations led to new strategies among dentists, namely, to extract teeth rather than preserving them to avoid possible focus of infection. Over time, evidence against focal infection theory was presented and special attention was paid to an article by Cecil in 1940. Patients with the same disease did not always show signs of focal infection and elimination of the foci did not always favour disease outcomes. In the 1950s, focal infection theory was abandoned.

Theories that oral health could influence general diseases regained interest when Mattila et al. (1989) described an association between dental health and myocardial infarction in a Finnish population. Overall dental infections (caries, periodontitis, endodontic lesions, and pericoronitis) were compared in a case-control study of 100 patients with acute myocardial infarction (AMI) and 100 randomly chosen subjects without AMI. Dental caries and periodontitis were more prevalent among patients with AMI than among controls. These differences remained significant even after adjusting for known risk factors for CAD, e.g., age, diabetes, and smoking. These published results became a starting point for a new era of interest for research on the CAD-periodontitis association.
Periodontitis

Periodontitis is the pathological manifestation of the host response against the bacterial challenges from the dental biofilm at the tooth and the gingival interface (Sanz and van Winkelhoff, 2011).

Due to inflammation and immune response, destructive processes involve the tooth’s supportive tissues: alveolar bone, root cementum, periodontal ligament, and gingiva. If untreated, this destruction proceeds over time, until the final consequence is tooth loss. The disease could be localised or wide spread in the dentition.

Bacterial plaques at the gingival margin initiate a classic inflammation with swelling, redness, and bleeding at the gingival margin with these clinical signs of gingivitis appearing in a time period of about seven days (Löe et al., 1965). When the bacterial plaque extends into the gingival sulcus, the connection between the junctional epithelium and the root surface disrupts and a pocket epithelium occurs. A deepened pocket reflects an environmental change from an aerobe to an anaerobe environment; the consequence is a shift in the microbial plaques – from a predominant gram positive flora that consists of various cocci and lactobacilli – to a subgingival flora that consists of gram negative rods and motile species (Listgarten and Helden, 1978).

Products from the biofilm – a community of various anaerobe and facultative aerobe species surrounded by gingival crevicular fluid – affect the pocket epithelium and the underlying connective tissue. Endothelial cells increase their expression of adhesion molecules and an inflammatory infiltrate occurs in the subepithelial connective tissue. Products, such as prostaglandin and destructive enzymes derived from bacteria or produced by cells in tissues, destroy the alveolar bone and with this bone destruction, the periodontal lesion is manifested (Page and Kornman, 1997).

Löe et al. (1965) found that periodontitis did not affect every subject, even though gingivitis, as an effect of neglected plaque control on tooth surfaces, developed in all subjects. Optimal oral plaque control that totally prevents gingivitis can’t occur in the total population. But improvement in oral hygiene has been described in studies from Jönköping County, Sweden (Hugoson and Norderyd, 2008). In 1973, mean plaque occurrence in the adult population (ages 20–70) was about 40% of the total number of available tooth surfaces, with slightly higher
occurrence among the oldest adults. Thirty years later, occurrence was less than 20%. Corresponding values were found for gingivitis.

Despite oral hygiene improvements, there were no or only minor changes in periodontitis prevalence. Moderately advanced periodontitis occurred in 28% of the population in 2003 and seemed to be unchanged over time. Severe periodontitis was found in 11% of the population; this reflects a slight reduction compared to the 13% prevalence 1973 (Hugoson et al., 2008). Although, periodontitis prevalence in Sweden in 2003 was comparable to prevalence found in studies of subjects without regular dental care and with high plaque levels (Baelum et al., 1986; Löe et al., 1986).

Recent data on periodontitis prevalence in Europe are inconsistent. König et al. (2010) concluded that actual epidemiological data on periodontal disease prevalence in epidemiological studies are heterogeneous and absent from several European countries. Eke et al. (2012) reported moderate periodontitis prevalence in 30% and severe periodontitis in 8.5% of the U.S. adult population in 2010. The number of studies that provide data on periodontitis prevalence over time is limited. From available data, Hugoson and Norderyd (2008) suggested that there might be a positive trend toward slightly decreasing severe periodontitis prevalence – at least in the Scandinavian countries.

Complexity of oral microflora

The oral cavity can be colonised by at least 600 bacteria species (Dewhirst et al., 2010) with a number exceeding 150 species in one subject. A healthy tooth pocket harbours a magnitude of $10^3$ microbes; a deep periodontal pocket with subgingival bacterial plaques harbours about $10^6$. These bacteria act together in a complex community surrounded by extracellular bacterial polymers and products derived from saliva and gingival exudates in a bio film (Darveau et al., 1997; Bernimoulin et al., 2003). Various species adhere to a surface or to each other. Nutrient products secreted by one species diffuse into the biofilm and can be useful for other species. A diffusion gradient for oxygen allows aerobe conditions on the surfaces and anaerobe in the deeper part of the pocket. The biofilm protect microbes against the host defence and antimicrobial substances such as antibiotics.
Several bacterial species have been closely associated with periodontitis, e.g., *P. gingivalis*, *A. actinomycetemcomitans*, *Prevotella intermedia*, *Bacteriodes forsythus* and spirochetes such as *Treponema denticola* (Socransky et al., 2002). Several other species are considered to be pathogens but their virulence factors are less pronounced: *Fusobacterium nucleatum*, *Campylobacter rectus*, *Eikenella corrodens*, *Selemonas* and *Eurobacterium* subspecies. Many species, such as *P. gingivalis*, can be detected (although in low numbers) in periodontally healthy subjects and subjects with gingivitis but no alveolar bone loss (Tanner et al., 1998). In a group of periodontally untreated men (Preus et al., 1995), 5% of all sites with periodontal health or gingivitis harboured *P. gingivalis*. Corresponding values for sites with 4-5 mm and ≥6 mm PPD were 28% and 40%, respectively. *A. actinomycetemcomitans* was not detectable in healthy sites but were equally distributed in sites with gingivitis and moderately advanced or advanced periodontitis (10%). Prevalence of *P. gingivalis* seems to increase with age, while *A. actinomycetemcomitans* is more prevalent among young subjects (Rodenburg et al., 1990). It has been showed that microbial species related to periodontitis decrease after periodontal treatment (Socransky and Haffajee, 1993; Haffajee et al., 1996).

*P. gingivalis* is a gram-negative, anaerobe, sackarolythic rod with several pathogenic characteristics; it has been associated with CAD (Andriankaja et al., 2011; Yakob et al., 2011). Endotoxin (the lipopolysaccharide [LPS] component of gram-negative bacterial cell walls) plays a key role in pathogenesis. LPS blocks expression of the e-selectin receptor on endothelial cells and thus inhibits leukocyte migration from the bloodstream into tissue. LPS (i) inhibits other bacterial species that can stimulate e-selectin expression and (ii) seems to block protein-1 chemotaxis and IL-8, an anti-inflammatory cytokine (Jain and Darveau, 2010).

*P. gingivalis* produces several factors, such as fibrinolysin and phospholipase A – plus enzymes that stimulate bone resorption, e.g., matrix metalloproteinases (MMPs). Arginine cysteine protease activity increases vascular permeability with increased inflow of exudates, which results in nutrients for biofilm. In active periodontal lesions, *P. gingivalis* prevalence increases and elevates levels of antibodies against *P. gingivalis*; antibodies can be measured in saliva in serum (Bostanci and Belibasakis, 2012).
Host response in periodontitis

Kinane et al. (2011) describe complex cellular and molecular mechanisms of host-microbial interactions. As a response to pathogenic subgingival microbiota, both innate and adaptive immune responses are activated (Figure 1). In the innate defence, mast cells activation, fibroblasts, and endothelial and epithelial cells results in unregulated cytokine secretion.

![Microbial stimuli](image)

*Figure 1. Host-response in periodontitis; modified from Kinane et al. 2011.*
Introduction

IL-8, IL-1β, TNF-α, and ICAM-1 promote vascular changes and due to IL-8, PGE2, and chemokines, polymorphonuclear leukocytes (PMNs) are activated and start to migrate. PMNs primarily have a protective role, but they can trigger production that drive tissue destruction, e.g., production of reactive oxygen species and various collagenases and proteases.

IL-1β, TNF-α, IL-6, and PGE2 stimulate the receptor activator of nuclear factor kappa-B ligand (RANKL), which is a member of the tumour necrosis factor (TNF) cytokine family, which is a ligand for osteoprotegerin (aka osteoclastogenesis inhibitory factor) – a key factor for osteoclast recruitment and activation.

In the adaptive immune response, antigen-presenting cells (such as Langerhans cells, macrophages and dentritic cells) activate native T cells. Depending on the type of presented pathogen, the immune system via a suitable cytokines profile reacts with cell-mediated immunity or antibody response.

INF-γ, IL-12, and IL-18 promote TH1 cells and by regulation of INF-γ, IL-5, and IL-13, this promotes cell-mediated immunity as the effect. IL-4 is capable to activate TH2 cells. Expression of IL-4, IL-21, and IL-13 promotes B-cell antibody production. T-regulatory cells regulate the processes, and TGF-β and IL-10 suppress the immune response.

Atherosclerosis

Normal histology of the arterial

Arteries are categorized as per size and characteristics: large (elastic arteries, medium or muscular and small (arterioles) with well-defined lumen surrounded by a three-layer coat (tunicae) that maintains vessel wall muscularity (Figure 2).

In tunica intima, the endothelium rests on a thin basal lamina. The subendothelial space contains proteoglycans and elastic collagenous fibres. Longitudinally oriented smooth muscle cells form a relatively thick band of elastic fibres.

The tunica media is the thickest layer with about 40 layers of circularly arranged smooth muscle cells with collagenous and reticular fibres – and no fibroblasts. The number of lamellae increases with age and with hypertension.
The *tunica adventitia* is a rather thin connective tissue layer that contains collagenous and elastic fibres. Fibroblasts constitute the main cell type, and many macrophages are present. Collagen in the adventitia prevents elastic arteries from stretching past their physiological limit during systole.

![Atherosclerosis stages, modified from Ross (1999).](Image)

*Figure 2. Atherosclerosis stages, modified from Ross (1999).*
Histopathological atherosclerosis stages are characterised by fatty streaks, fibrofatty lesion, and fibrous plaque. Ross (1999) described atherosclerosis as an inflammatory disease. It progresses in steps over time – or more likely in bursts (Bruschke et al., 1989). Due to the chronic inflammatory response, a cascade of events results in migration and proliferation of arterial smooth muscle cells and increases accumulation of lipid and lipoproteins beneath the endothelium. In the first step, endothelial dysfunction leads to monocyte migration and development of early intimal lesion with fatty streaks. If the offending agent continues, the inflammatory response results in cytokines production from leukocytes that initiate migration and proliferation of smooth muscle cells. These produce an extracellular matrix, and the lesion expands to an intermediate, fibrofatty lesion, with multiplication of smooth muscle cells, connective tissue, macrophages, and T lymphocytes. Remodelling processes result in the advanced lesion with formation of a fibrous cap that has many smooth muscle cells, which produce extracellular matrix proteins, elastic fibres, and proteoglycans. As the lesion enlarges, it intrudes into the vessel wall and the arterial lumen narrows. Blood flow decreases, which may lead to clinical symptoms. In stable cases, plaque is stabilised, and the atheroma (with its lipid-rich core) is covered by a relatively thick, firm, fibrous cap with preserved vessel lumen. In vulnerable plaque, a large lipid pool (with many inflammatory cells) is covered by a thin fibrous cap. Changes in the surface of this fibrous cap could cause erosions or ruptures, which (i) leads to formation of thrombus that compromises blood flow at a local site and (ii) in the worst case, causes sudden death (Figure 2).

Atherosclerosis, a consequence of inflammation and immune response

The atherosclerosis pathogenesis is complicated. It involves a series of inflammatory action in the innate and adaptive host immune response (Libby, 2002). Under normal conditions (when blood passes through the arterial vessel), the endothelium resists leukocyte adhesion. This condition changes when pro-inflammatory risk factors for atherosclerosis occur (e.g., hypertension and high cholesterol levels – especially modified LDL and hyperglycaemia).

Recruitment of inflammatory cells from the blood stream is one of the first steps in the normal artery transition to an early atherosclerotic lesion. An up-
regulation of various adhesion molecules on the endothelial cells (where VCAM-1 plays a major role) promotes leukocyte binding to the endothelium. In a directed migration, the leukocytes enter the intima of the vessel wall by diapedesis between the endothelial cells at their junctions. Chemoattractant cytokines (chemokines) (e.g., MPC-1 that is produced by vascular cells as a response to modified lipoproteins) stimulate monocytes recruitment. During diapedesis, monocytes release matrix metalloproteinase (MMP-9), which degrade collagen type IV, and this enables monocytes to enter into the atherosclerotic lesion. Resident in the intima, monocytes change morphologically into macrophages. Expression of the SRA and CD36 scavenger receptors (on their surfaces) is enhanced in presence of antigens, e.g., oxidized LDL and heat shock proteins (HSP). Modified lipoproteins are taken up into the macrophages and accumulated in cytoplasmic drops in the macrophage, which leads to lysosomal degeneration. Cholesterol esters accumulation in the cytoplasm morphologically transforms macrophages into foam cells, which are characteristic for early atherosclerotic lesions with fatty streaks (Libby, 2002).

Monocytes in atherosclerotic plaques express toll-like receptors on their surfaces as a response to several molecules, e.g., HSP and oxidized LDL. Inflammatory responses are activated when monocytes secrete growth factors and cytokines – including tumour necrosis factor-α (TNF-α) and interleukin-1β (II-1β).

Secreted cytokines initiate migration and proliferation of smooth muscle cells. The monocytes, which originated from the innate immune response, are the most plentiful leukocytes in all atherosclerosis stages. Mast cells can populate the adventitia but appear in fewer numbers. They deregulate and release cytokines and proteases such as MMPs. Activated mast cells can induce endothelial cell death (Libby et al., 2009).

T cells dominate the adaptive immune response in atherosclerosis (Packard et al., 2009). They enter the intima by binding to adhesion molecules, including VCAM-1, as a response to a chemoattractant (in the same way as monocytes). In the arterial intima, the T cells encounter antigens; one of them is oxidised LDL. The most frequently occurring T cells in the atherosclerotic lesion are cytotoxic T cells (Th1 CD4+). Antigen-presenting cells (as dendritic cells or B-lymphocytes with MHC class II molecules on their surfaces) present the antigen.

Macrophages are activated in the TH1 pathway. Phagocytosis (including release of MMPs and gelatinases) causes collagen breakdown and thus a weakened fibrous cap that covers atherosclerotic plaque. Extracellular antigens
enhance the TH2 pathway and activate the B-lymphocytes to produce specific antibodies against, for example, oxidized LDL.

**Plaque disruption**

Libby (2002) describes three types of physical plaque disruption:

1. *Fracture of the plaque’s fibrous cap.* In athermanous plaque, an over-expression of collagens (MMP-1, -8, and -13) seems to degrade the fibrous cap. When plaque ruptures, extracellular matrix components are exposed to cells in the circulatory system where they get in contact with coagulation proteins in the blood stream.

2. *Superficial erosions.* Collagen and the von Willebrand factor activate platelets, and the wound-healing cascade is triggered. Often, a limited mural thrombus forms. In the healing process, resorption of the thrombus response leads to an inflammatory response with release of anti-inflammatory cytokines and growth factors such as PDGF. Deposition of smooth muscle cells and collagen forms a more fibrous cap. Mechanisms of superficial erosions in microscopic areas of the thin endothelial layer also occur. In these areas, subepithelial collagen is exposed. When the von Willebrand factor activates this collagen, platelet adhesion and activation is stimulated, which results in platelet thrombus.

3. *Disruption of newly formed micro vessels in the atherosclerotic plaques.* These newly formed vessels are fragile and easily disrupted in the lesion, where they cause thrombosis inside the lesion. This leads to thrombin generation and cleaving of fibrin that is caused by secretion of TGF-β from activated platelets, which trigger proliferation and migration of smooth muscle cells that synthesize interstitial collagen.

**Wound healing process**

Wound healing is a non-linear process, which integrates with dynamic, interactive processes that involve soluble mediators, extracellular matrix, and cells (Clark, 1996). Wound healing starts with inflammation. In areas of injured
tissue, blood vessels rupture. The Hageman factor increases permeability and bradykinin increases vessel dilation. By activation of the complement system, neutrophiles and monocytes are recruited and releases active products. Histamine and leucotrienes are released from mast cells. Platelets adhere to connective tissue, and an aggregate of platelets blocks injured vessel walls. Varying proteins and soluble factors are released from platelet granules, e.g., PDGF and TGF-β; both initiate chemotaxis of fibroblasts. Re-epithelialisation is promoted by TGF-β and TGF-α within hours after an injury. Epithelial cells change their phenotype and become motile due to loss of their desmosomes. When the barrier is restored, then the original phenotype is established.

Fibrin and integrins facilitate cell-to-cell contact and act as a reservoir for cytokines. Neovascularisation is complex. Cells in the tissue, e.g., macrophages, produce factors that stimulate endothelial cells to release plasminogen and procollagenase, which promotes endothelial migration.

In the last wound-healing stage, tissue is modulated to scar tissue. Connective tissue gradually replaces granulation tissue. Fibroblast apoptosis transforms the tissue into denser connective tissue with fewer cells.

**Hepatocyte growth factor**

The hepatocyte growth factor (HGF) is a protein involved in wound healing. Mesenchymal cells secrete HGF, which regulates angiogenesis, vascular permeability, cell migration, reepithelialisation, and other wound healing processes (Conway et al., 2006).

HGF was initially filtrated from rat serum and found to initiate growth of hepatocytes (Nakamura et al., 1984). In the 1990s, it was found to influence cell growth and cell mortality for various epithelial cells and to play a key role as a mediator via an epithelial-mesenchymal interaction in wound healing, tissue regeneration, and morphogenesis (Nakamura, 1991). Bussolino, et al. (1992) described angiogenic factors and regenerative effects from HGF. Over time, HGF was found to be structurally different from other growth factors.

The mesenchymal epithelial transition (MET) factor is the receptor for HGF, and the HGF-MET pathway plays key roles in epithelial morphogenesis. Almost all epithelial, endothelial, and erythroid progenitor cells express the c-MET, an HGF receptor (Nakamura et al., 2011).
c-MET expression seems to be higher in acute wounds (compared to chronic), while the HGF is similar, which indicates a more active role in acute wound healing (Conway et al., 2007).

Elevated HGF serum levels were associated with acute coronary syndrome (ACS) (Matsumori et al., 1997; Sato et al., 1999; Shimada et al., 2002; Lenihan et al., 2003). Susen et al. (2005) proposed that HGF serum level is an independent predictor of clinical outcome after percutaneous coronary intervention (PCI). The HGF was associated with periodontitis. HGF levels in saliva were correlated with number of deep pockets (Ohshima et al., 2002, Wilczynska-Borawska et al., 2006) and HGF levels in gingival crevicular fluid were higher in periodontally compromised sites (Nagaraja and Pradeep, 2007). In vitro, cysteine proteinase – an arginine-specific gingipain from P. gingivalis – stimulates human gingival fibroblasts that secrete HGF (Uehara et al., 2005).

**Periodontitis-CAD association**

A large number of epidemiological studies describe an association between oral health and cardiovascular disease (CVD). In this thesis, Table (1-4):

1. Displays associations between number of teeth and CVD.
2. Describes studies that report the oral health-CVD association.
3. Lists studies that reported clinical periodontal variables (gingivitis, bleeding on probing [BOP], and probing pocket depth [PPD]) associated with CVD.
4. Presents studies on the alveolar radiographic bone level-CVD association.

The studies’ varying designs make them suitable for various research purposes.
## Introduction

### Table 1. Studies one number of teeth - CVD.

<table>
<thead>
<tr>
<th>CVD variable</th>
<th>Tooth variable</th>
<th>p-value</th>
<th>Adjustment</th>
<th>Author</th>
<th>Yr</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke/TIA</td>
<td>TLN</td>
<td>0.0016</td>
<td>Age, smoking, HT, diabetes, cholesterol</td>
<td>Elter et al.</td>
<td>'03</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CARTOID ARTERY PLAQUE</td>
<td>NLT</td>
<td>0.05</td>
<td>Age, gender, diabetes, systolic BP, serum cholesterol, race-ethnicity, education</td>
<td>Desvarieux et al.</td>
<td>'03</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CARTOID ARTERY PLAQUE</td>
<td>NT</td>
<td>0.05</td>
<td>Age, gender, diabetes, systolic BP, serum cholesterol, race-ethnicity, education</td>
<td>Desvarieux et al.</td>
<td>'04</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>MI</td>
<td>NLT</td>
<td>-</td>
<td>Age, smoking, hypertension, social factors</td>
<td>Paunio et al.</td>
<td>'03</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CAD ≤16 teeth</td>
<td>-</td>
<td></td>
<td>Age, smoking, HT, diabetes, serum cholesterol, BMI</td>
<td>Elter et al.</td>
<td>'04</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>MI, self-reported CAD</td>
<td>NT</td>
<td>0.03</td>
<td>Age, gender, smoking</td>
<td>Holmlund et al.</td>
<td>'06</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CARTOID ARTERY IMT</td>
<td>NT</td>
<td>0.016</td>
<td>Age, sex, smoking, BMI, blood glucose, triglycerides, cholesterol, C-reactive protein, leukocyte count, BP, Framingham risk score</td>
<td>Holmlund et al.</td>
<td>'12</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CAD</td>
<td>NT</td>
<td>0.02</td>
<td>Age, diabetes, smoking, BMI, education</td>
<td>Buhlin et al.</td>
<td>'05</td>
<td>Case-control</td>
</tr>
<tr>
<td>ACS</td>
<td>NLT</td>
<td>0.02</td>
<td>Age, gender, smoking, diabetes, HT, and BMI</td>
<td>Buhlin et al.</td>
<td>'11</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD incidence</td>
<td>TLN</td>
<td>-</td>
<td>Age, gender, diabetes, smoking, serum cholesterol, HT</td>
<td>Morisson et al.</td>
<td>'99</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>CVD incidence ≥9 teeth</td>
<td>Missing</td>
<td>0.024</td>
<td>Age gender, smoking, BMI, systolic BP, socioeconomic status</td>
<td>Tu et al.</td>
<td>'07</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>CAD</td>
<td>NT</td>
<td>&lt;0.0001</td>
<td>Age, gender, smoking</td>
<td>Holmlund et al.</td>
<td>'10</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>MI</td>
<td>NT at baseline</td>
<td>0.03</td>
<td>Age, smoking</td>
<td>Joshipura et al.</td>
<td>'96</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>CAD</td>
<td>TLN</td>
<td>ns</td>
<td>Age, gender, social status, smoking, alcohol, BP, cholesterol, diabetes, physical activity, psychological factors</td>
<td>Hjoel et al.</td>
<td>'01</td>
<td>Longitudinal</td>
</tr>
</tbody>
</table>

intima-media thickness = IMT; transient ischemic attack = TIA; toothlessness = TLN; number of teeth = NT; number of lost teeth = NLT; blood pressure = BP; hypertension = HT
### Table 2. Studies on tooth infection/oral health - CVD.

<table>
<thead>
<tr>
<th>CVD</th>
<th>Tooth-related variable</th>
<th>p-value</th>
<th>Adjustments</th>
<th>Author</th>
<th>Yr</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD</td>
<td>Overall tooth infections</td>
<td>&lt;0.001</td>
<td>Age, serum cholesterol, smoking, hypertension, BMI,</td>
<td>Mattila et al.</td>
<td>'93</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CAD</td>
<td>Overall tooth infections</td>
<td>ns</td>
<td>Age, social factors, smoking, hypertension, serum lipids</td>
<td>Mattila et al.</td>
<td>'00</td>
<td>Case-control</td>
</tr>
<tr>
<td>MI</td>
<td>Over all dental infections</td>
<td>&lt;0.01</td>
<td>Age, smoking, hypertension, diabetes, education, social class, BMI cholesterol, glucose.</td>
<td>Montebugnoli et al.</td>
<td>'04</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD death</td>
<td>Overall dental infections</td>
<td>0.053</td>
<td>Age, smoking, BMI, diabetes, hypertension, education level</td>
<td>Karhunen et al.</td>
<td>'06</td>
<td>Case-control</td>
</tr>
<tr>
<td>MI</td>
<td>Overall tooth infections</td>
<td>-</td>
<td>Age, gender, socioeconomic status, smoking, hypertension, previous MIs, diabetes, BMI serum lipids</td>
<td>Mattila et al.</td>
<td>'95</td>
<td>Longitudinal</td>
</tr>
</tbody>
</table>
Table 3. Studies on the clinical periodontal variables - CVD.

<table>
<thead>
<tr>
<th>CVD</th>
<th>Periodontal variable</th>
<th>p-value</th>
<th>Adjustments</th>
<th>Author</th>
<th>Yr</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-R heart attack</td>
<td>AL ≥ 3 mm</td>
<td>0.02</td>
<td>Age, smoking, HT, serum cholesterol, BMI, social factors</td>
<td>Arbes et. al.</td>
<td>'99</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>IMT ≥ 1 mm</td>
<td>AL ≥ 3 mm ≥ 30% of sites</td>
<td>0.001</td>
<td>Hypertension, serum cholesterol, diabetes, smoking</td>
<td>Beck et al.</td>
<td>'01</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CVD</td>
<td>S-R gingival inflammation</td>
<td>0.0017</td>
<td>Age, smoking, diabetes, social status</td>
<td>Buhlin et al.</td>
<td>'01</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>TIA/Stroke</td>
<td>N sites AL ≥ 3 mm</td>
<td>0.003</td>
<td>Age, smoking, HT, diabetes, serum cholesterol</td>
<td>Elter et al.</td>
<td>'03</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CAD</td>
<td>AL ≥ 3 mm ≥ 10% of sites</td>
<td>0.0001</td>
<td>Age, smoking, HT, diabetes, serum cholesterol, BMI</td>
<td>Elter et al.</td>
<td>'04</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Coronary artery calcification</td>
<td>AL ≥ 3 mm ≥ 10% of sites</td>
<td>ns</td>
<td>Age, smoking, HT, diabetes, serum cholesterol, BMI</td>
<td>Nakib et al.</td>
<td>'04</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Cartoid artery plaque</td>
<td>% sites attachment level ≥ 4 mm</td>
<td>0.05</td>
<td>Sex, age, diabetes, systolic BP, serum cholesterol race-ethnicity, education</td>
<td>Desvarieux et. al.</td>
<td>'04</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>ACS</td>
<td>% teeth cal ≥ 5 mm</td>
<td>0.002</td>
<td>Age, smoking, diabetes, family history of CAD</td>
<td>Gotsman et al.</td>
<td>'07</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>CAD-related death</td>
<td>Periodontal status (no disease/gingivitis/ PD 4-6 mm/PD&gt;6 mm)</td>
<td>ns</td>
<td>Age, gender, education level, smoking, HT, hypercholesterolemia, diabetes</td>
<td>Tuominen et al.</td>
<td>'03</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>Angina pectoris/MI</td>
<td>≥ 5 mm PPD</td>
<td>0.0001</td>
<td>Age, gender, smoking, alcohol intake, diet, HT, hyperlipidemia, physical activity, diabetes</td>
<td>Geerts et al.</td>
<td>'04</td>
<td>Case-control</td>
</tr>
</tbody>
</table>

intima-media thickness = IMT; transient ischemic attack = TIA; self-reported = S-R; attachment loss = AL; blood pressure = BP; hypertension = HT

.... Table 3 continued on next page.
Table 3 continued …

<table>
<thead>
<tr>
<th>CVD</th>
<th>Periodontal variable</th>
<th>p-value</th>
<th>Adjustments</th>
<th>Author</th>
<th>Yr</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI</td>
<td>Proportion ≥6 mm PPD</td>
<td>0.05</td>
<td>Age, gender, diabetes, cholesterol</td>
<td>Persson et al.</td>
<td>'03</td>
<td>Case-control</td>
</tr>
<tr>
<td>AMI</td>
<td>BOP</td>
<td>0.01</td>
<td>Age, gender, social status</td>
<td>Renvert et al.</td>
<td>'04</td>
<td>Case-control</td>
</tr>
<tr>
<td>cMTA/IMT</td>
<td>≥5 mm PPD</td>
<td>0.003/</td>
<td>Age, gender, smoking, BMI, diabetes, hypertension, plasma cholesterol</td>
<td>Söder et al.</td>
<td>'05</td>
<td>Case-control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>No. of ≥4 mm PPD</td>
<td>0.006</td>
<td>Age, gender, smoking, diabetes, cholesterol</td>
<td>Buhlin et al.</td>
<td>'05</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD</td>
<td>≥4 mm PPD</td>
<td>0.047</td>
<td>Perfect matched</td>
<td>Tabrizi et al.</td>
<td>'07</td>
<td>Case-control</td>
</tr>
<tr>
<td>BOP</td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD/ACS</td>
<td>≥6 mm PPD</td>
<td>0.049</td>
<td>Age, gender, smoking, diabetes, HT, BMI</td>
<td>Buhlin et al.</td>
<td>'11</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD</td>
<td>Periodontal index score</td>
<td></td>
<td>Age, gender, race, social factors, smoking, alcohol, systolic BP, total cholesterol, diabetes, BMI, physical activity</td>
<td>deStefano et al.</td>
<td>'93</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>CAD</td>
<td>Gingivitis</td>
<td>-</td>
<td>Age, gender, province, diabetes, smoking, serum cholesterol, HT</td>
<td>Morrison et al.</td>
<td>'99</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>MI/CVD death</td>
<td>S-R periodontitis</td>
<td>ns</td>
<td>Age, smoking, HT, BMI, cigarette &amp; alcohol use, physical, parental MI history</td>
<td>Howell et. al.</td>
<td>'01</td>
<td>Longitudinal</td>
</tr>
</tbody>
</table>

Intima-media thickness = IMT; transient ischemic attack = TIA; self-reported = S-R; attachment loss = AL; blood pressure = BP; hypertension = HT
Introduction

Table 4. Studies on the radiographic alveolar bone loss - CVD.

<table>
<thead>
<tr>
<th>CVD</th>
<th>Tooth-related variable</th>
<th>p-value</th>
<th>Adjustments</th>
<th>Author</th>
<th>Yr</th>
<th>Study design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid plaque thickness</td>
<td>Overall ≥50% bone loss</td>
<td>0.003</td>
<td>Age, gender, smoking, diabetes, hypertension, cholesterol</td>
<td>Engbertson et al. '05</td>
<td>'05</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>AMI</td>
<td>Bone loss ≥4 mm in ≥50% of the teeth</td>
<td>0.01</td>
<td>Age, gender, diabetes, cholesterol</td>
<td>Persson et al. '03</td>
<td>'03</td>
<td>Case-control</td>
</tr>
<tr>
<td>AMI</td>
<td>Proportion bone loss ≥4 mm</td>
<td>&lt;0.001</td>
<td>Smoking</td>
<td>Renvert et al. '04</td>
<td>'04</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD</td>
<td>Vertical bone defects</td>
<td>0.06</td>
<td>Age, gender, smoking, diabetes, cholesterol</td>
<td>Buhlin et al. '05</td>
<td>'05</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD</td>
<td>Mean alveolar bone level</td>
<td>0.003</td>
<td>Age, smoking, diabetes</td>
<td>Geismar et al. '06</td>
<td>'06</td>
<td>Case-control</td>
</tr>
<tr>
<td>CAD/ACS</td>
<td>ABL</td>
<td>0.025</td>
<td>Age, gender smoking, diabetes, hypertension, BMI</td>
<td>Buhlin et al. '11</td>
<td>'11</td>
<td>Case-control</td>
</tr>
<tr>
<td>ACS recurrence</td>
<td>Extent of bone loss ≥4 mm</td>
<td>0.001</td>
<td>Age</td>
<td>Renvert et al. '10</td>
<td>'10</td>
<td>Longitudinal</td>
</tr>
</tbody>
</table>

acute coronary syndrome = ACS; acute myocardial infarction = AMI; alveolar bone level = ABL
Cross-sectional studies

A cross-sectional study design is observational; it describes a subset of the total population at a specific time point – prospective or retrospective. This design provides values that indicate disease prevalence and is suitable for studying covariations of two diseases in a population. Associated risk factors can be studied, but cross-sectional study designs cannot prove cause-related factors. An association between CAD and tooth health in men was first described in Finnish studies reported in the 1990s. Atherosclerosis found on coronary angiography was more common among patients who had dental infections of various kinds that were diagnosed on panoramic radiographs (Mattila et al., 1993). Patients with angina pectoris or who had myocardial infarction (MI), had fewer teeth than healthy subjects (Paunio et al., 1993).

In the US, a subset of the National Health and Nutrition Examination survey (NHANES III) Arbes et al. (1999) reported an increased OR for self-reported heart-attack with higher numbers of sites with attachment loss ≥3 mm.

The Atherosclerotic Risk in Communities study investigated the aetiology and natural history of atherosclerosis and CVD in four US communities. Several studies reported results from the subsets with more than 6000 subjects (female and male). Beck et al. (2001) found an odds ratio (OR) of 1.3 for having carotid artery intima thickness of ≥1 mm if having severe periodontitis that was defined as ≥3 mm attachment loss at ≥30% of the sites. An OR of 1.3 was found for stroke and transient ischemic attack and an OR of 1.4 for edentulous (Elter et al., 2003). The combination of high attachment loss and less than 16 natural teeth gave elevated odds for prevalent CAD (Elter et al., 2004), although Nakib et al. (2004) did not find coronary artery calcification associated with periodontitis.

The Oral Infections and Vascular Disease Epidemiology study is a population-based cohort study on random subjects (female and male) living on Manhattan without stroke history or MI. Desvarieux et al. (2003) reported subclinical atherosclerosis measured on high-resolution ultrasound, significantly associated with tooth loss. An association between tooth loss, high percentage of sites with attachment level (AL) ≥4 mm and subclinical atherosclerosis was found in men but not in women (Desvarieux et al., 2004). Overall alveolar bone loss ≥50% on panoramic radiographs was associated with carotid plaque thickness (OR 3.64), compared to subjects experiencing less bone loss.
Introduction

(Engebretson et al., 2005). The overall burden of pathogens associated with periodontitis was related to carotid intima media thickness (cIMT) (Desvarieux et al., 2005).

For CAD patients in Israel, the percentage of teeth with a ≥5 mm clinical attachment level was associated with CAD severity in terms of multiple vessel disease – compared to one vessel disease (Gotsman et al., 2007), although the study failed to prove any association between various periodontitis severity levels and CAD – probably because almost all patients had severe periodontitis.

Cross-sectional studies in Sweden reported data similar to US data. Buhlin et al. (2002) found self-reported gingival inflammation associated with CVD in both sexes (OR of 1.6). Number of teeth was associated with MIs and self-reported MIs that were associated with clinically diagnosed periodontitis and bone level measured via radiographs (Holmlund et al., 2006). Later, Holmlund and Lind (2012) suggested that (i) there is a exposure-response relationship between number of teeth and cIMT and (ii) tooth loss is a good cIMT predictor.

Case-control studies

Case-control studies retrospectively identify patients with CAD and compare them to control subjects without CAD. The results reflect the investigated group, although there is risk of misinterpretations when transferring results to other populations. But if inclusion criteria are correct and if controls are randomly chosen and well-matched and properly checked for confounders, then case-control studies provide valuable information.

Results from the Mattila (1989) case-control study were followed up with another study (Mattila et al., 2000) that compared subjects with and without clinically and angiographically diagnosed CAD. This study failed to prove any relation between periodontitis and CAD, possibly due to selection-bias concerning various ages in the group. But in another study (Karhunen et al., 2006), poor oral health due to dental infections (e.g., periodontal vertical pockets and furcation involvements, caries, and periapical lesions that were determined post mortem with panoramic tomography) was associated with sudden cardiac death diagnosed via autopsy. Dental infections were associated with risk of death due to CAD – especially in men < 50 years.

Periodontitis classified according to alveolar bone level on radiographs, number of deep periodontal pockets, and clinical periodontal variables were
related to CVD, although the definition for periodontitis varies. Geerts et al. (2004) reported severe periodontitis correlated with angina pectoris and MI (OR 6.5). Montebagnoli et al. (2004) found these factors associated with overall periodontal variables: (i) acute myocardial infarction (AMI) less than six month before the study and (ii) at least 50% stenosis in at least one of the coronary arteries. These findings are aligned with Buhlin et al. (2005) who reported an associations between CAD and periodontitis in females with severe CAD. In this study, ≥10 sites with >4 mm PPD, number of teeth, number of vertical bone defects, and overall mean marginal bone level on radiographs were associated with CAD. The association remained after adjustment for CAD risk factors.

The ultimate case-control study should be a twin study, but it is difficult to find sufficient subjects for such a study. Tabrizi et al. (2007) reported one study that included 10 monozygotic twin pairs. Presence of CAD within the twin pairs was associated with periodontitis. Twins with CAD had significantly more BOP and ≥4 mm PPD. An OR of 1.17 for a twin to belong to the CAD group was reported, although a logistic regression may not be a favourable statistical analysis method in such a small group.

Söder et al. (2005) suggested that periodontitis is an independent predictor for (i) carotid intima-media thickness (cIMT), with an OR of 4.64, and for (ii) the carotid intima-media area (cIMA), with an OR of 5.2.

Evidence of bone loss around several teeth was suggested as a valuable factor for predicting future AMI (Persson et al., 2003) and CAD (Geismar et al., 2006). Risk of having AMI and periodontitis increased with percentage of teeth with bone loss of >4 mm. In older patients, no associations were found. Renvert et al. (2004) reported that the combination of bone loss of ≥4 mm on ≥50% of the teeth and clinical periodontal variables had the highest association with AMI in the total sample, but on an individual level, radiographically measured bone loss seemed to be the best parameter.

**Longitudinal cohort studies**

In longitudinal prospective cohort studies, groups of individuals are followed over time; they receive repeated examinations. Consequently, disease progression can be followed over time. These results are usually more valid and reliable than results from case-control studies.
Introduction

Mattila et al. (1995) found poor oral health associated with new fatal and nonfatal cardiac events in a group of 214 CAD patients, who were followed more than 7 years.

de Oliveira et al. (2010) reported increased risk of cardiovascular events in subjects with poor oral hygiene (self-reported tooth brushing habits). In this eight-year follow up, subjects who responded with never/rarely brushing teeth (n=538 of total 11869 subjects) had a hazard ratio of 1.7 for CVD – compared with subjects who responded with brushing teeth once or twice a day.

In a classic study by DeStefano et al. (1993), a sample of 9760 subjects from the previously described NHANES was followed over 8–11 years. Periodontitis was found as an increased risk for CAD with relative risk (RR) of 1.72 in men ages <50 and RR of 1.25 in the total sample. Renvert et al. (2010) reported that an additional ACS event was associated with periodontitis (OR of 3.6).

In a study of 1147 CAD and healthy subjects, who were followed more than 8 years, (Beck et al., 1996) associated alveolar bone level at baseline with cumulative incidence of CAD, with an OR of 1.5 for total CAD events (i.e., angina, non-fatal MI, and CAD-related death). No adjustment was made for smoking habits.

The number of teeth was associated with CAD in several longitudinal studies (Morrison et al., 1999; Joshipura et al., 2003; Tu et al., 2007).

Morrison et al. (1999) found edentulousness associated with CAD with RR of 1.90 (and gingivitis with RR of 2.15) in a retrospective design that included a baseline sample of 10,368 subjects without self-reported CAD, where cumulative CAD incidence was followed up after 20 years.

In a Joshipura et al. (2003) study, self-reported number of teeth was associated with fatal or nonfatal MI and sudden death. RR for CAD was 1.76 if having 10 or fewer teeth, compared to 25 teeth or more; here, 44,119 men without previously diagnosed CAD at baseline were followed up after four years. Since revascularisation procedures (PCI and CABG) were excluded as endpoints (outcomes), this ought to have had an impact on results.

Tu et al. (2007) report on an exceptionally long, 57-year follow-up. Tooth loss, a categorical variable, was associated with death from CVD, with an increased risk for CVD and a hazard ratio (HR) of 1.35 – if missing 9 or more teeth at baseline. The study population consisted of students ages ≤30 (n=12,223) at baseline. Over time, 509 deaths due to CVD (405 of those from CAD) were reported.
Holmlund and Lind (2011) described a dose-dependent relationship between the number of teeth and CAD-related mortality. Causes of mortality over 12 years were studied in a sample of 7688 patients, who were referred for periodontal treatment at a specialist clinic, and 886 randomly selected subjects from the same area in Sweden. The number of remaining teeth was significantly related to mortality due to CVD and CAD. The authors suggested that the number of teeth could be used as an indicator for oral health in association with CVD.

Some studies, however, failed to prove a CAD-periodontitis association (Howell et al., 2001; Hujoel et al., 2001; Tuominen et al., 2003). Self-reported periodontal disease was not an independent predictor of CAD over 12 years (Howell et al., 2001) and long-term elimination of all dental infections, about toothlessness did not reduce CAD risk compared to subject with periodontitis in a retrospective 17 years follow up study by (Hujoel et al., 2001). Tuominen et al. (2003) found no association between death in CAD and periodontal status (4 groups; no disease, gingival inflammation, 4-6 mm PPD, <6 mm PPD) over 12 years (6527 subjects 30-60 years at baseline). Confounding factors (especially cigarette smoking) were proposed to explain associations found in other studies.

**Meta-analysis**

Meta-analysis is considered to have high evidence value. The study design offers a review of original studies included after distinct criteria, aiming to conclude results. Bahekar et al. (2007) reported that CAD prevalence and incidence increase in subjects with periodontitis. Periodontitis increased the risk for CAD in cohort studies with a RR of 1.14 (1.074–1.213) and in case-control studies with an OR of 2.22 (1.59-3.117). Patients with <10 teeth had increased risk for CAD by 1.24 (1.14-1.36). Cross-sectional studies showed higher CAD prevalence in subjects with periodontitis – compared to periodontally healthy subjects with an OR of 1.59 (1.329-1.907), CI 95%.

In conclusion, epidemiological studies suggested an association between CVD and CAD and number of teeth and periodontal disease in terms of (i) clinical periodontal variables and (ii) bone loss on radiographs. While the studies suggested an association, they did not prove case-related factors.
Possible explanatory factors for the periodontitis-CAD association

Systemic impact from oral microflora

In a meta-analysis, Mustapha et al. (2007) studied the association between periodontal disease and elevated systemic bacterial exposure and CAD. Periodontal disease was strongly associated with CAD – compared to subjects without periodontitis, with an OR of 1.75. The authors suggested that periodontitis-related, systemic, bacterial, exposure levels could be one biological explanation for the role of periodontitis in atherosclerosis.

Pathogens associated with periodontitis were found in atherosclerotic plaques (Haraszthy et al., 2000). In 50 endarterectomies, from 50 men with carotid stenosis, 44% of the atheromas were positive for one or more periodontal-associated species, namely, T. forsythia (T. forsythia; previously Tanerella forsythia; formerly Bacteroides forsythus) was found in 30%; P. gingivalis, in 26%; A. actinomycetemcomitans, in 18%; and Prevotella intermedia, in 14%.

Figuero et al. (2011) examined 42 atheromatous plaques and reported:
- 78% prevalence of P. gingivalis
- 67% prevalence of A. actinomycetemcomitans
- 62% prevalence of T. forsythia
- 55% prevalence of Eikenella corrodens
- 50% prevalence of Fusobacterium nucleatum
- 9% prevalence of Campylobacter rectus

Renvert et al. (2006) found that the oral bacterial load of several periodontitis-related pathogens (including P. gingivalis, T. forsythia, and Treponema denticola) was associated with ACS in 161 patients and in 161 matched control subjects. Spahr et al. (2006) showed that the total periodontal pathogen burden was associated with risk of CAD with an OR of 1.92 in a sample of 263 patients with angiographically confirmed stable CAD and 526 population-based age- and gender-matched controls without a CAD history. The number of A. actinomycetemcomitans yielded an OR for CAD risk of 2.7.
Several authors (Stein et al., 2009; Andriankaja et al., 2011) suggested that *P. gingivalis* might be a possible link between oral health and atherosclerosis. In case-control studies, *P. gingivalis* was found to be (i) a significant predictor of AMI with an OR of 13.6 (Stein et al., 2009) and (ii) associated with increased risk for MI with an OR of 1.62 (Andriankaja et al., 2011). Holmlund et al. (2011) found antibody level against *P. gingivalis* related to MI in 100 patients with MI – compared to 100 matched controls. MI patients had impaired oral health in terms of >4 mm PPD, >20% BOP, and periodontal bone loss scores on full-mouth X-rays. The authors suggested that *P. gingivalis* might be a link between oral health and CVD.

Yakob et al. (2011) demonstrated a relationship between periodontitis and the carotid intima-media area (cIMA). In this case-control study, exploratory factors for the increased cIMA values were: male gender, hypertension, body mass index, lower socioeconomic status, and periodontitis (OR of 4.2). Prevalence of *P. gingivalis* (OR of 7.6) and of *Prevotella nigrescens* (OR of 4.1) was also associated with cMCA.

In a study of 20 periodontitis and 20 atherosclerosis patients, Choi et al. (2011) indicated that periodontitis (as an infectious disease) could be linked to atherosclerosis as an autoimmune disease. Immunoreactive epitopes of *P. gingivalis* and heat shock protein (HSP60) were found in all 20 CAD patients (all had signs of periodontitis) who underwent surgical intervention for atheromatous plaques, compared to 30% of the 20 periodontitis patients without CAD.

**Thrombocytes activation**

Bacteria can bind to the receptors of the platelets, directly or via bridging ligands. A cascade of signal leads to platelet activation and aggregation, via binding to fibrinogen in plasma. This capacity was demonstrated for bacteria such as *Helicobacter pylori*, *Chlamydia pneumonia*, and *P. gingivalis*, which are described most often (Fitzgerald et al., 2006). *P. gingivalis* causes in vitro platelet aggregation in mice (Sharma et al., 2000). P-selectin (a marker of activated endothelial cells and thrombocytes) is enhanced in periodontitis patients. In vitro stimulation with *P. gingivalis* and *A. actinomyctecomitans* results in P-selectin expression on surfaces of platelets and endothelial cells (Assinger et al., 2011). The platelet-activating factor in serum was shown to be higher in subjects with
periodontitis and in subjects with CAD, compared to healthy controls (Chen et al., 2010).

**Endothelial dysfunction**

Higashi et al. (2008) suggested that systemic inflammation, maintained by chronic periodontitis, may be one cause of endothelial dysfunction that fosters CAD. Compared to healthy controls, male periodontitis patients, but without known risk factors for CVD, showed endothelial dysfunction. The association between periodontitis and endothelial dysfunction was further assessed in a case-control study that compared CAD patients with periodontitis and CAD patients without periodontitis (Higashi et al., 2009). The authors speculated that the observed difference in enhanced endothelial dysfunction in CAD patients with periodontitis may be caused by decreased nitric oxide production, which leads to impaired nitric oxide bioavailability. Mechanisms behind the observed association between endothelial function are not fully understood (Li et al., 2011).

**Systemic inflammation markers**

An elevated pre-operative CRP >3 mg/l, is a risk factor for long-term cardiac morbidity and non-fatal MI (Padayachee et al., 2009). C-reactive protein (CRP) is an acute-phase protein that is mainly synthesized by the liver in response to inflammation. CRP is a pattern recognition molecule that binds to specific molecular configurations exposed on cells during cell death or found on the surfaces on pathogens. CRP has ability to bind to phosphocholine on microbes and thus assists complement binding and phagocytosis by macrophages, as part of the innate immune response. Increasing plasma concentrations of IL-6 (predominantly produced by macrophages) result in increased CRP levels as a response (Black et al., 2004).

Elevated plasma in systemic inflammation markers (including CRP, IL-6, circulating leukocytes, and neutrophils) were associated with periodontitis (Loos, 2000; Loos et al., 2005). Noack et al. (2001) found >3 mm clinical attachment loss associated with CRP ≥3 mg/l – compared to periodontally healthy controls (AL ≤2 mm) with an OR of 4.0. Accordingly, increased IL-18 levels (OR of 6.6) and fibrinogen (OR of 8.7) were significantly associated with periodontitis (Buhlin et
al., 2009). In a Monteiro et al. (2009) study, leukocyte and neutrophile counts and IL-6 were higher in periodontal patients than in controls. In contrast, high-sensitivity serum C-reactive protein (hs-CRP) was not related to periodontal status in patients with MI (Persson et al., 2005). But in a systematic review from 2008 (Paraskevas et al., 2008), the authors concluded that CRP plasma levels are elevated in subjects with periodontitis – compared to control subjects without periodontitis.

Serum lipid levels

A higher cholesterol level was suggested as a link between CAD and periodontitis. Katz et al. (2001) reported an association between severe periodontitis and hypercholesterolemia in patients with angiographic evidence of CAD or MI. Griffiths and Barbour (2010) suggested a more proatherogenic lipid profile in periodontitis patients, which consequently promotes increased lipid peroxidation and thus atherosclerosis. Ligature-induced periodontitis in a rats increased lipid peroxidation in serum (Ekuni et al., 2009). Higher serum antibody levels against oxLDL occurred in periodontitis patients, compared to healthy controls (Monteiro et al., 2009). But in subjects with low HDL, a relationship was found between presence of deep pockets and subclinical atherosclerosis (Ylöstalo et al., 2010). A population-based study (that consisted of never smokers only), failed to prove an association between serum lipid levels and periodontal infections in normal-weight subjects. But low HDL levels were associated with periodontitis in obese subjects (Saxlin et al., 2008).

Periodontal treatment effect on systemic inflammation

Many epidemiological studies reported a positive CAD-periodontitis association. Inflammation factors related to CAD are found in serum from periodontitis patients. If this association is causal, it is conceivable that periodontitis treatment could reduce risk of CAD or/and risk of recurrence of CAD. In systematic reviews, authors concluded that periodontal treatment results in moderate reduction of CRP serum levels (Paraskevas et al., 2008) and that periodontal treatment in systemically healthy subjects improves systemic inflammation and endothelial dysfunction (Tonetti, 2009).
Introduction

Elevated IL-6, TNF-α, E-selectin, and D-dimers (a decomposition product from fibrin) serum levels could be measured between one and seven days after scaling and root planning in patients with severe periodontitis, which demonstrates that periodontal treatment influenced inflammation, endothelial function, and coagulation (D’Aiuto et al., 2007).

In a group of 18 men with AMI, oral hygiene and subgingival scaling were performed to measure changes in systemic inflammation after three months (Montebugnoli et al., 2005). A decrease was found for CRP and oxLDL serum levels.

Accordingly, extraction of all teeth due to severe periodontitis reduced CRP serum levels and fibrinogen plus blood cell and platelets counts (Taylor et al., 2006). Buhlin et al. (2009) showed an effect on several inflammation factors one year after successful treatment of severe periodontitis. An increased HDL serum level occurred as well as a decrease in LDL and lower IL-18 and INF-γ levels. Secondary prevention regarding periodontal treatment in patients with earlier CAD events (Offenbacher et al., 2009) reduced high-sensitivity CRP (hs-CRP) levels in non-obese patients with hs-CRP >3 mg/l levels.

Shared risk factors – confounders

CAD and periodontitis share many risk factors, and these are possible confounders to the association between the diseases. This fact must be considered when evaluating the association between two diseases. Table 5 summarises known risk factors for CAD and periodontitis. Many studies support a positive correlation between CAD and periodontitis – even after controlling for known risk factors. When evaluating validity of the studies’ designs, inclusion and exclusion criteria are crucial.
Table 5. Risk factors or risk indicators associated with atherosclerosis and periodontitis.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Atherosclerosis</th>
<th>Periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-modifiable</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gender</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Family history</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Race</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Modifiable</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Obesity</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypertension</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Dental plaque</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diabetes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Infections</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Psychological stress</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Tobacco smoking is particularly discussed, and it is known to be a causal factor in CAD (Bucerius et al., 2011) and periodontitis (Bergström, 2004; Martinez-Canut et al., 1995).

Compared to non-smokers, smokers had a 2.95 higher RR for non-fatal MI. Risk decreased gradually over time in former smokers but remained more than 20 years after smoking cessation (Teo et al., 2006). The observed RR from smoking on periodontitis was reported to be 2–4, compared to non-smokers – depending on numbers of cigarettes per day (Grossi et al., 1994). Risks for periodontitis increase if subjects smoke more than 10 cigarettes per day. Smokers tend to have a more severe and wide-spread attachment loss with deeper periodontal pockets (Haffajee and Socransky, 2001). Several possible biological explanations were suggested to explain the impact of tobacco smoking on periodontitis, i.e., changed inflammatory cytokine profiles (de Heens et al., 2009) and a shift toward a more Th2 response (de Heens et al., 2009). Although smoking is a shared risk factor, the CAD-periodontitis association remains in most published studies, even after controlling for the effect of smoking or in the few studies that include never smokers only.

Diabetes triples the risk for CAD (Stamler et al., 1993) and poorly controlled diabetes doubles the risk for periodontitis (Grossi et al., 1994). Severe periodontitis is more prevalent in subjects with long-duration diabetes, especially if the diabetes has caused complications (Thorstensson and Hugoson, 1993). Several mechanisms for this relationship were proposed. Diabetes affects function of immune cells such as neutrophiles, monocytes, and macrophages. Chemotaxis and phagocytosis may be impaired, and an effect of this could be
inability of the immune response to deal with subgingival bacteria (Mealey and Oates, 2006). Periodontitis may have an impact on the metabolic control of diabetes, and a correlation between periodontal health and glycemic control in type-2 diabetes has been shown. In type-1 diabetes, this is more controversial and not yet proven (Lakschevitz et al., 2011).

Obesity and overweight constitute a considerable risk factor for CAD (Eckel and Krauss, 1998). An overall OR of 1.35 was reported for obesity as a risk factor for periodontitis (Chaffee and Weston, 2010). In a meta-analysis (Suvan et al., 2011), odds ratios in relation to body mass index categories were:

- Obesity, an OR of 1.81
- Overweight, an OR of 1.27
- Combination of obesity and overweight, an OR of 2.13.

Falagás and Kompoti (2006) suggested that adipose tissue has an impact on immune response in several immune mediators, which makes subjects more susceptible to infections.

CAD prevalence increases with age (Ferrari et al., 2012). Periodontitis prevalence increases with age due to accumulated attachment loss over time and effects of aging. In a group of well-functioning elderly (ages 80+), severe periodontitis was found in more than half of the subjects (Holm-Pedersen et al., 2006). It seems that tooth loss and bone loss severity increase with age, independent of frequency of visits to dentists (Renvert et al., 2011).

Male gender is a risk factor for CAD (Buceri et al., 2011). A gender difference in periodontitis prevalence was reported, although the effect is comparatively small compared to other risk factors (Shiau and Reynolds, 2010a). A biological explanation why men are slightly more susceptible to periodontitis than women could be an effect from sex steroids on various immunological parameters involved in inflammation, i.e., inflammatory cytokines levels (Shiau and Reynolds, 2010b).

Psychological factors, such as stress and coping behaviours, may have an impact on CAD and periodontitis. Anxiety has been related to recurrent events in CAD (Grewal et al., 2011). Monteiro da Silva et al. (1996) reported that reduced general immune response is an effect of chronic psychological stress. Negative life events, depression, and anxiety seem to be over-represented in periodontitis patients (Monteiro da Silva et al., 1996; Axtelius et al., 1998; Johannsen et al., 2005). Socransky and Haffajee (1992) suggested that psychological factors have an effect on environmental factors that favour bacteria related to periodontitis – via the impact of psychological stress on microcirculation in gingival tissues.
A possible explanation for the co-existents between CAD and periodontitis may be a common underlying genetic background. Polymorphism of the pro-inflammatory cytokine IL-1 gene was associated with severe periodontitis and CAD (Geismar et al., 2008; Goteiner et al., 2008). A described shared genetic susceptibility locus on human chromosome 9p21.3 may be a start to understand the complexity of why periodontitis and CAD seems to co-vary in the population (Schaefer et al., 2009).
Rationale for the studies

The literature and contemporary studies suggest a CAD-periodontitis association when it comes to clinical and radiological periodontal parameters and inflammation, although at present the exact mechanisms are not fully understand. This thesis reports on studies of associations between CAD and periodontitis.
OBJECTIVES

General aim
The overall aim of studies described in this thesis was to investigate the CAD-periodontitis association.

Specific aims
Specific aims of the studies were to:

- Compare periodontal conditions in patients with CAD and subjects without a history of CAD.

- Study whether or not periodontal status influences outcomes in known CAD over an 8-year period.

- Study whether or not concentrations and biological activity of hepatocyte growth factor in serum from patients with severe CAD differs depending on occurrence of periodontitis.

- Study concentrations and biological activity of hepatocyte growth factor in serum, saliva, and gingival crevicular fluid in healthy subjects with or without periodontitis.
HYPOTHESES

The studies’ hypotheses were:

- Periodontal disease is more prevalent in patients with coronary artery disease compared to subjects without CAD (I).

- Patients with CAD and periodontal disease have worse heart disease outcomes over time – compared to patients without periodontal disease (II).

- The biological activity of HGF is reduced in patients with CAD (III).

- The biological activity of HGF is reduced in patients with periodontal disease (IV)
MATERIAL

Ethical considerations

The University of Linköping ethics committee approved the studies’ protocols. [Dnr] 00-103 (I), 2011/429-32 (II), 98-426 (III) and 2010/307-31 (IV). Participants were assured confidentiality of the collected data and given opportunities to withdraw from the studies at any time. All participants provided written, informed consent.

Selection of patients and controls

Table 6 presents an overview of the subjects (I–IV). CAD patients (I-III) were recruited from consecutive patients referred to the Department of Cardiology, University Hospital, Linköping, Sweden, for coronary angiography because of known or suspected angina pectoris between 2000 and 2004. Patients were inhabitants in Östergötland or Jönköping counties with a total population about 760,000.

Table 6. Patients and controls in studies I-IV.

<table>
<thead>
<tr>
<th>Sty</th>
<th>Patients (n) &amp; inclusion</th>
<th>Controls (n) &amp; inclusion</th>
<th>Mean age, SD cases/controls</th>
<th>Female (n) cases/controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>161. Significant coronary stenosis, who underwent PCI or CABG at cardiac clinic, University Hospital.</td>
<td>162. Randomly selected with no CAD history, recruited from Sweden’s population register. Matched for age, gender, community.</td>
<td>61±8.9/62±8.8</td>
<td>29/29</td>
</tr>
<tr>
<td>II</td>
<td>126. From study I.</td>
<td>121. From study I.</td>
<td>68±8.9/69±9.0</td>
<td>24/20</td>
</tr>
<tr>
<td>III</td>
<td>36. Significant coronary stenosis who underwent PCI.</td>
<td>56. Healthy blood donors, matched for age and gender.</td>
<td>60±7.4/59±7.3</td>
<td>10/15</td>
</tr>
<tr>
<td>IV</td>
<td>30. Severe periodontitis, systemically healthy referred to Periodontology Dept.</td>
<td>30. Systemically healthy, age- and gender-matched subjects without periodontitis.</td>
<td>55±11.6/55±11.8</td>
<td>13/13</td>
</tr>
</tbody>
</table>

percutaneous coronary intervention = PCI; coronary artery by-pass graft = CABG
Exclusion criteria were diabetes mellitus, rheumatoid arthritis, malignant diseases, acute infections, and concurrent medication with glucocorticoids. All patients – ages ≤75, who had significant coronary stenosis, ≥50% stenosis in the coronary artery lumen in one or several of the coronary arteries, and who subsequently underwent percutaneous coronary intervention (PCI) or coronary artery by-pass graft (CABG) – were included consecutively.

Control subjects (I, II) were recruited from the Swedish population database. Each control subject was matched by age, gender, and community to one CAD patient. Controls were excluded if they had CAD or if any of the other exclusion criteria were present; the same applied to the study group. In study III, age- and gender-matched healthy blood donors were recruited as controls regarding the blood analysis.

Patients with severe periodontitis (IV) were recruited from consecutive patients referred to the Department of Periodontology, Centre for Oral Rehabilitation, Folkandvården, Östergötland, Sweden.

Inclusion criteria were healthy subjects with chronic periodontitis. Patients were included if (i) bone loss displayed on radiographs exceeded loss of 1/3 of the root length on most teeth and (ii) deep pockets with bleeding on probing were present. They should not have received periodontal treatment at a specialist clinic.

The control group (IV) consisted of healthy active or retired co-workers at the Centre for Oral Rehabilitation without any signs of periodontitis – matched for age and gender.
METHODS

Clinical periodontal examination

Studies I-IV. Periodontal conditions were determined as per Lindhe and Nyman (1975). These clinical parameters were recorded:

- **Number of remaining teeth** – number of teeth, excluding third molars.
- **Dental plaque** – presence or absence of visible plaque at the gingival margin on four surfaces (buccal, lingual, mesial, and distal) of each tooth corresponding to scores 2 and 3 of the plaque index system of Silness & Löe (1964), calculated in per cent of the total number of available sites (PII%).
- **Probing pocket depth** – the distance from the gingival margin to the bottom of the probed pocket, determined using a manual periodontal probe (HuFriedy PCP 11). Depth was determined to the nearest whole mm at four surfaces on each tooth. Pockets were recorded if they were 3 mm or deeper at buccal and lingual sites and 4 mm or deeper at mesial and distal sites as per Lindhe & Nyman (1975).
- **Bleeding on probing** – presence of bleeding after probing at the bottom of the pocket. The percentage of the total number of sites that bled was recorded.

Radiographic examination and periodontal disease severity rating

Studies I-IV. A set of full-mouth intra-oral radiographs (I, II, IV) including bitewing projections, was taken for each subject using a standardised parallel technique (Eggen, 1969). This included an individualised number of intra-oral radiographs taken with an Eggenhällare. Extra oral panoramic radiographs were taken on all included CAD patients in study III. The alveolar bone level was measured (I, II) at all proximal sites in millimetres along the root surface from the cemento-enamel junction (or from the most apical part of an interfering restoration) to the most coronal level at which the width of the periodontal ligament space was considered normal.
Methods

In study:
1. Two calibrated periodontists took all measurements; the periodontists were blinded. Measurements were then compared, and in cases of disagreement, consensus was reached.

II-IV. The author of this thesis took all measurements

A classification as per severity of the periodontal disease experience was made (I-IV) on each subject based on clinical and radiographic findings. Criteria include a modification, including all remaining teeth, of the index by Hugoson & Jordan (1982), namely, Group:
1. Healthy or almost healthy gingival units, normal alveolar bone height, and ≤12 bleeding units in the molar-premolar regions.
2. Gingivitis, normal alveolar bone height, and >12 bleeding gingival units in the molar-premolar regions.
3. Alveolar bone loss around most teeth, not exceeding 1/3 of normal bone height.
4. Alveolar bone loss around most teeth, ranging between 1/3 and 2/3 of normal bone height.
5. Alveolar bone loss around most teeth, exceeding 2/3 of normal bone height and presence of angular bony defects and furcation defects.

Questionnaire

Studies I-IV. All subjects filled in a questionnaire that covered a wide variety of items related to oral factors, general health, lifestyle, and social environment. In study II, several questions regarding CAD were added; they are based on work by Rose et al. (1977):
- Pain or discomfort in your chest (yes/no)?
- Do you get this pain or discomfort when you walk uphill or hurry (yes/no)?
- Do you get this pain or discomfort when you walk at an ordinary pace on the level (yes/no)?
- When you get any pain or discomfort in your chest what do you do (stop or slow down/continue at the same pace/take nitro-glycerine)?
Data from hospital medical records

Studies I-III. Baseline data concerning CAD diagnosis and treatment modalities among CAD patients were taken from medical records at the Cardiac Clinic, University Hospital Linköping (studies I-III). In study II, data on the cardiac endpoints in terms of MI or revascularisation procedures (PCI/CABG) or cardiac death during the eight years between baseline periodontal examination and periodontal re-examination were collected. These data in the medical records were noted: chest pain, angina pectoris, non-STEMI, STEMI, cardiac failure, new coronary angiography, and treatment with PCI or CABG or no invasive treatment.

Cause of death

Study II. After ethical approval from the National Board of Health and Welfare, Stockholm, cause of death was obtained from Sweden’s death index for all deceased subjects when death was due to CAD.

Subgingival plaque

Studies III and IV. Subgingival microbial samples were collected from four sites, the deepest, which bleed on probing in each quadrant, or in healthy subjects, from all mesial sites at the first premolars. Supragingival plaque was removed, and the root surface dried by air. The bacterial sample was collected by insertion of a sterile endodontic study point into the periodontal pocket for 20 seconds and then transferred to a sterilised test tube. In study III, the presence of P. gingivalis was analyzed by PCR (Slots et al., 1994). In study IV, samples were processed in the Department of Oral Microbiology and Immunology, University of Gothenburg, Sweden and analysed for their content of 18 bacterial species, using checkerboard DNA-DNA hybridisation technology (Socransky et al., 1994) (IV).
Methods

Blood, saliva, and gingival crevicular fluid samples

Studies III and IV. Peripheral venous blood was collected from CAD patients (study III) before and after angiography and PCI (24 hours, 1 month, 6 month, 12 months) and from healthy blood donors in one occasion (controls in study III).

In study IV, peripheral venous blood from healthy periodontitis patients and from periodontally healthy controls was collected before, three minutes after, and 20 minutes after subgingival debridement (IV). Blood samples were centrifuged, and the serum was collected and stored on ice until frozen. Before subgingival debridement, five ml of non-stimulated whole saliva was collected (study IV). Gingival crevicular fluid (GCF) was collected from four sites and selected the same way as for the subgingival plaque samples (study IV). The volumes were measured using the Periotron 8000 (Oraflow Inc, New York, U.S.A.), calibrated after the protocol as per Chapple et al. (1999).

HGF concentration

Studies III and IV. The HGF concentration was determined using an ELISA kit (Quantikine Human HGF immunoassay, minimum detectable limit: 0.04 ng/mL; R&D Systems, Minneapolis, MN) as per manufacturer’s instructions. The measurements of the samples were performed in duplicate at 450 nm using an ELISA reader (Expert 96; Asys Hitech GmbH, Eugendorf, Austria), and calibrated using the recombinant human HGF reference samples and standards that were provided in the ELISA kit.

HGF activity

Studies III and IV. The biological activity of HGF was analysed with surface plasmon resonance (SPR) an optical technology that can determine the affinity of a protein for ligands or epitopes (Liedberg et al., 1995, Nayeri et al., 2005). Biological activity was assessed by measuring binding affinity to HSPG (Sigma-Aldrich, St. Louis, MO, USA) and the binding to c-MET (R&D Systems, Minneapolis, MN, USA). SPR-based assessment of the binding profile of HGF to HSPG may sensitively distinguish HGF variants with different biological activities and has been used for evaluation of the quality of endogenous HGF.
Methods

(Nayeri et al., 2008). SPR measurements and ligand immobilisation procedures were conducted in a fully automatic Biacore 1000 instrument (GE-Healthcare GmbH, Uppsala, Sweden).

Statistical analysis

The student T-test determined significant differences in normally distributed quantitative data between two independent groups (II, IV). The Mann–Whitney test (I, III, IV) was used when criteria for T-test was not fulfilled. The Kruskal-Wallis (III, IV) was used to compare means between more than two groups. A significance test, for comparing two proportions with inclusion of continuity correction, evaluated differences in proportions between two groups (I, II). Data in study III were normally distributed after logarithmation and analysed by repeated measures of ANOVA, followed by the Neuman-Keuls Post Hoc test. The chi-square test determined if there was an association between categorical variables, i.e., CAD-related endpoints and periodontal disease experience at baseline (II). Stepwise logistic regression analysis (I) evaluated differences between test and control groups based on predictor variables. Results are reported as means ± standard deviation (SD) or as medians. A p-value <0.05 was considered to be statistically significant. To determine the number of participants in the test and control groups necessary for giving the study results significance, a power analysis with 80% power at a 5% significance level was performed in pre-study planning (I). Intra- and inter-examiner reliability studies were done (I).

SPSS® 13.0 (I, III) or 18.0 (II, IV) software package (SPSS for Windows NT 4.0, SPSS Inc., Chicago, IL) and Graph Pad Prism® V 5 (San Diego, CA) (III, IV) were used for calculations and statistical analyses.
RESULTS

Study I

We compared periodontal conditions in CAD patients and healthy matched control subjects. CAD patients had significantly fewer teeth, lower alveolar bone level, and higher PII%, number of 4-6 mm PPD and BOP%. No difference was found regarding >6 mm PPD.

Table 7. Clinical parameters for CAD patient group (n=161) and control group (n=162) at baseline and re-examination: number of remaining teeth, % surfaces with visible plaque at gingival margin, BOP, number of PPDs, and mean bone level. Standard deviation noted in brackets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAD patients, Mean (SD)</th>
<th>Range</th>
<th>Control, mean (SD)</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of teeth</td>
<td>22.7 (5.5)</td>
<td>5-28</td>
<td>24.6 (4.1)</td>
<td>8-28</td>
<td>0.002</td>
</tr>
<tr>
<td>Plaque %</td>
<td>46.0 (26.0)</td>
<td>0-100</td>
<td>39.0 (21.5)</td>
<td>0-90</td>
<td>0.05</td>
</tr>
<tr>
<td>BOP %</td>
<td>27.0 (19.6)</td>
<td>0-100</td>
<td>21.0 (16.3)</td>
<td>0-68</td>
<td>0.009</td>
</tr>
<tr>
<td>4-6 mm PPD</td>
<td>14.0 (12.4)</td>
<td>0-68</td>
<td>10.7 (10.3)</td>
<td>0-49</td>
<td>0.007</td>
</tr>
<tr>
<td>&gt;6 mm PPD</td>
<td>0.6 (1.7)</td>
<td>0-16</td>
<td>0.4 (1.0)</td>
<td>0-6</td>
<td>0.328</td>
</tr>
<tr>
<td>Bone level (mm)</td>
<td>3.0 (1.0)</td>
<td>1.1-7.6</td>
<td>2.6 (0.8)</td>
<td>1.0-5.9</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Prevalence of severe periodontitis was higher in patients with CAD – compared to healthy control subjects. Twenty-five per cent of CAD patients were categorised into periodontal disease experience groups 4 & 5, compared to 8% of controls (Figure 3). Percentages without bone loss (groups 1 & 2) were the opposite, 24% among CAD patients and 40% among controls.
**Results**

*Figure 3.* Distribution of CAD patients and healthy controls as per periodontal disease experience group (Hugoson & Jordan 1982).

<table>
<thead>
<tr>
<th></th>
<th>CAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=161</td>
<td>n=162</td>
</tr>
<tr>
<td>25% Group 1 + 2</td>
<td>24% Group 3</td>
<td>8% Group 4 + 5</td>
</tr>
<tr>
<td>51% Group 1 + 2</td>
<td>52% Group 3</td>
<td>40% Group 4 + 5</td>
</tr>
</tbody>
</table>

Groups. 1: Healthy or almost healthy gingival units, normal alveolar bone height, and ≤12 bleeding units in molar-premolar regions. 2: Gingivitis, normal alveolar bone height, and >12 bleeding gingival units in molar-premolar regions. 3: Alveolar bone loss around most teeth not exceeding 1/3 of normal bone height. 4: Alveolar bone loss around most teeth ranging between 1/3 and 2/3 of normal bone height. 5: Alveolar bone loss around most teeth exceeding 2/3 of normal bone height and presence of angular bony defects and furcation defects.

**Study II**

We investigated if periodontal status at baseline influenced differences in long-term CAD-related outcomes. Differences in periodontal variables between the re-examined 126 CAD patients and the 121 control subjects remained during the eight-year observation period. Refer to *Table 8.*

CAD-related endpoints (i.e., MI, PCI, CABG or death) were found in 26 CAD patients. Three control subjects had CAD endpoint during the observation period. In the group of 161 CAD patients included at baseline, no significant differences were found regarding CAD-related endpoints in relation to baseline periodontal disease experience group ($p=0.7$). In the total sample of 323 subjects (161 CAD patients and 162 controls) a higher correlation existed, however not statistically significant ($p=0.052$), between periodontal disease experience groups at baseline and experienced CAD related endpoints. Refer to *Table 9.*
Results

Table 8. Clinical parameters for CAD patient group (n=126) and control group (n=121) at baseline and re-examination: number of remaining teeth, % surfaces with visible plaque at gingival margin, BOP scores, number of PPDs, and bone level. Standard deviation noted within brackets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAD baseline n=126 Mean (SD)</th>
<th>Range</th>
<th>Control baseline n=121 Mean (SD)</th>
<th>Range</th>
<th>p-value*</th>
<th>CAD re-exam n=126 Mean (SD)</th>
<th>Range</th>
<th>Control re-exam n=121 Mean (SD)</th>
<th>Range</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. teeth</td>
<td>23.3 (5.2)</td>
<td>5–28</td>
<td>24.7 (4.1)</td>
<td>8–28</td>
<td>0.018</td>
<td>22.3 (5.8)</td>
<td>1-28</td>
<td>24.2 (4.4)</td>
<td>4-28</td>
<td>0.006</td>
</tr>
<tr>
<td>Plaque (%)</td>
<td>43.2 (23.7)</td>
<td>0–100</td>
<td>37.6 (21.5)</td>
<td>0–90</td>
<td>0.054</td>
<td>31.0 (21.0)</td>
<td>1-87</td>
<td>24.4 (18.5)</td>
<td>0-93</td>
<td>0.01</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>24.3 (17.1)</td>
<td>0–82</td>
<td>21.0 (16.0)</td>
<td>0–68</td>
<td>0.125</td>
<td>12.1 (14.8)</td>
<td>0-69</td>
<td>6.6 (10.9)</td>
<td>0-69</td>
<td>0.001</td>
</tr>
<tr>
<td>4-6 mm PPD</td>
<td>14.2 (12.2)</td>
<td>0–59</td>
<td>10.3 (10.3)</td>
<td>0–49</td>
<td>0.008</td>
<td>12.7 (12.0)</td>
<td>0-59</td>
<td>9.2 (10.6)</td>
<td>0-70</td>
<td>0.016</td>
</tr>
<tr>
<td>&gt;6 mm PPD</td>
<td>0.5 (1.7)</td>
<td>0–16</td>
<td>0.4 (1.0)</td>
<td>0–6</td>
<td>0.595</td>
<td>0.7 (2.9)</td>
<td>0-29</td>
<td>0.4 (1.8)</td>
<td>0-17</td>
<td>0.321</td>
</tr>
<tr>
<td>Bone level mm</td>
<td>3.0 (1.0)</td>
<td>1.1–6.5</td>
<td>2.5 (0.8)</td>
<td>1.0–5.9</td>
<td>0.001</td>
<td>3.0 (1.0)</td>
<td>1.1-5.5</td>
<td>2.5 (0.9)</td>
<td>1.0-6.5</td>
<td>0.042</td>
</tr>
</tbody>
</table>

*Student t-test (CI 95%)

Table 9. Subject distribution in the periodontal disease experience index-based groups when accounting for CAD-related endpoints between baseline and re-examination in (i) 26 CAD patients out of 161 at baseline and (ii) 29 subjects out of a total baseline sample of 323 subjects (161 CAD patients and 162 controls).

<table>
<thead>
<tr>
<th>Index-based groups</th>
<th>1 &amp; 2</th>
<th>3</th>
<th>4 &amp; 5</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD endpoints** (i)</td>
<td>5 (19.2)</td>
<td>14 (53.8)</td>
<td>7 (26.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Not CAD endpoints</td>
<td>33 (24.4)</td>
<td>75 (55.6)</td>
<td>27 (20.0)</td>
<td></td>
</tr>
<tr>
<td>CAD endpoints** (ii)</td>
<td>5 (17.2)</td>
<td>15 (51.7)</td>
<td>9 (31.0)</td>
<td>0.052</td>
</tr>
<tr>
<td>Not CAD endpoints</td>
<td>96 (32.8)</td>
<td>152 (51.9)</td>
<td>45 (15.4)</td>
<td></td>
</tr>
</tbody>
</table>

*The association between endpoint status and periodontal disease experience is tested with Chi-square.

Percentages of subjects are noted in parentheses/brackets.

**Coronary artery disease (CAD), myocardial infarction (MI), percutaneous coronary intervention (PCI), and coronary artery bypass graft (CABG)
Study III

We investigated HGF concentration and biological activity in relation to periodontal conditions in CAD patients. Before PCI, HGF concentrations in serum were significantly higher in CAD patients compared to controls (i.e., healthy blood donors), 1.11±0.37 ng/ml compared to 0.78±0.29 ng/ml (p<0.001). In patients without periodontal bone loss (gingivitis group), HGF concentration increased 24 hours after PCI, in parallel with increased biological activity, measured as the binding capacity to HSPG measured in response units (Figure 4). One month after PCI, biological activity peaked and thereafter, the concentration decreased to values near initial values. In CAD patients with periodontitis (moderate or severe), only small fluctuations occurred in HGF values, in the concentration and the biological activity. Observed differences in HGF biological activity between CAD patients with or without periodontitis one month after PCI was statistically significant, p<0.05. No differences in HGF concentrations were found among CAD patients with varying periodontal status or with positive/negative prevalence of *P. gingivalis.*
Results

**Figure 4.** Serum concentration (HGF ng/ml) and biological activity (HSPG RU) of HGF patients with CAD before (pre), 24 hours, 1 month, 6 and 12 months after PCI intervention in relation to periodontal status and prevalence or no prevalence of *P. gingivalis* (response units=RU). Repeated ANOVA measurements were used for statistical analysis; data reported as mean ± SD.
Study IV

We investigated the concentration and biological activity of HGF in medically healthy subjects with periodontitis. Periodontal patients showed increased concentrations of HGF (compared to periodontally healthy controls) in saliva ($p<0.001$), in GCF ($p<0.0001$), and in serum ($p<0.001$) samples.

The serum concentration of HGF did not differ between sampling times (collected before, 3 minutes, and 20 minutes after subgingival debridement).

The biological activity of HGF measured as the binding affinity to HSPG and c-MET was significantly reduced in saliva ($p<0.0001$) and GCF samples ($p<0.0001$ for HSPG and $p<0.01$ for c-MET) from the periodontitis patients, compared to the healthy controls. In serum samples, no significant differences were found in the binding affinity to HSPG, while the c-MET binding were increased in the patient group ($p<0.05$) 3 minutes after subgingival debridement.

Figure 5 displays an overview of HGF concentrations (HGF (ng/ML) and biological activity via HSPG binding (RU) and c-MET binding (RU).

The amount and frequency distribution of periodontal pathogens were higher in patients with severe periodontitis – compared to healthy subjects ($p<0.01$).

All patients (100%) had one or several of the 12 studied bacteria species associated to periodontitis that were analysed from subgingival plaques – compared to 60% of the healthy controls. No differences were found regarding HGF concentration and the binding affinity to c-MET and HSPG as per prevalence of the 12 studied periodontal pathogens.
Results

Figure 5. HGF concentrations (HGF ng/ml) and HGF biological activity, HSPG (HSPG (RU)) and c-MET binding (c-MET (RU)); in serum, saliva and gingival crevicular fluid in patients with severe periodontitis (n=30) and periodontally healthy controls (n=30).

Serum

![Serum HGF and HSPG binding plots](image)

Saliva

![Saliva HGF and c-MET binding plots](image)

... Figure 5 continued on next page.
Results

Figure 5 continued . . .

Gingival crevicular fluid

The concentration of HGF (ng/ml) in saliva (A), gingival crevicular fluid (GCF) (B) and serum (C) measured with ELISA, and the binding affinity to the HGF receptors heparan sulphate proteoglycan (HSPG) (D, E and F) and c-Met (G, H and I) analysed by surface plasmon resonance (RU) in patients with severe periodontitis (patients) and healthy controls (control). Kruskal-Wallis and Mann-Whitney U-test was used for statistical analysis and the medians are presented.

****p<0.0001, ***p<0.001, **p<0.01, *p<0.05.
DISCUSSION

The main findings reported in this thesis support the CAD-periodontitis association. There is a significant difference in CAD between individuals with and without periodontitis. However, high prevalence of severe periodontitis in CAD patients unchanged during an eight year follow-up, did not influence CAD outcomes (I, II). One factor involved in wound healing and tissue regeneration, namely, the hepatocyte growth factor (HGF) seems to be of importance in these patient categories (III, IV). As in other chronic inflammations (Nakamura and Mizuno, 2010), HGF concentrations were higher in CAD patients (III) and in patients with severe periodontitis (IV). HGF-reduced biological activity in CAD patients with periodontitis (III) might indicate that periodontitis and/or CAD have an impact on healing potential and could be one of the mechanisms in the association between the two diseases.

In study I, CAD patients had severe periodontitis more often compared to subjects without CAD; this is aligned with findings in other case-control studies (Geerts et al., 2004; Montebugnoli et al., 2004; Renvert et al., 2004; Buhlin et al., 2005; Geismar et al., 2006; Buhlin et al., 2011), and it strengthens the epidemiological finding that CAD and periodontitis seem to be covariates in the population. In study I, 25% of the CAD patients had severe periodontitis compared to 8% of the controls, which in the CAD-group is a higher prevalence compared to the reported prevalence of periodontitis in the general Swedish population ages 40+ (Hugoson et al., 2008). The relative low number of subjects with severe periodontitis among control subjects in study I, compared to prevalence reported in another study (Hugoson et al., 2008), might reflect the effect from chance but could also reflect the inclusion criteria. Subjects in study I had to be free from a history of CAD and from other inflammation-related diseases. The exclusion criteria were selected to avoid confounding from diseases known to have impact on CAD and periodontitis. The study I control group could be seen as medically healthier than the general population in the same age category because exclusion criteria in the group were (i) diabetes, rheumatoid arthritis, malignancy, and (ii) intakes of corticosteroid drugs. This might explain why these subjects also had better periodontal health compared to the general Swedish population. Periodontitis, however, might have a larger impact on CAD-related disease if the CAD patients also have diabetes.
We did not exclude smokers (I, II, III, IV), although smoking is one of the major risk factors for CAD and periodontitis (Bucerius et al., 2011; Bergström, 2004). The power analysis (I) was based on CAD and periodontitis prevalence in the general population, including smokers. A study on never smokers would have been preferable but in that case, the time period for recruiting subjects would have to be longer than the actual three years and a larger sample size would be required.

Effects from severe periodontal disease on CAD patients were studied in logistic regression analysis adjusted for age and smoking. An OR of 5.74 (2.07-15.90) was found for CAD patients – compared to controls in periodontal disease groups 4 and 5, which indicates that severe periodontitis at least doubles risk for CAD. This finding is aligned with an overall OR of 2.22 (1.59-3.117) reported for case-control studies (Bahekar et al., 2007), and it supports the relevance of studying periodontitis in relation to CAD.

Results from study I (case-control design) apply only to the investigated groups, so comparison with other groups is problematic. However, study I has strength. All CAD patients had documented significant stenosis with at least 50% occlusion of the coronary artery (confirmed by coronary angiography). A specialist in cardiology recruited the patients. Two experienced calibrated periodontists did the periodontal examinations and ratings, and an inter-examiner study (I) showed kappa values by 87% and intra-examiner high reproducibility over 90% (I).

Periodontal disease severity was based on alveolar bone level on radiographs and on clinical periodontal variables. These findings are aligned with other studies (Renvert et al., 2004; Buhlin et al., 2005): higher overall alveolar bone loss, higher numbers of 4-6 mm periodontal pockets and higher BOP scores among CAD patients.

The observed differences in periodontal disease prevalence and severity in study I was consistent over time (II). If the follow-up period had been longer, CAD outcome might have been different, because periodontitis cases most often progresses slowly. Reduced plaque and gingivitis levels might indicate that all examined subjects were informed about their periodontal status and that recommendations about suitable treatments were given.

The number of teeth (or number of lost teeth) has been suggested as a predictor associated with CAD (Buhlin et al., 2005; Holmlund et al., 2006; Tu et al., 2007; Holmlund et al., 2010). In studies I and II, a difference in number of teeth was observed between CAD patients and controls at baseline and at eight-year, follow-up examinations (p=0.002 in study I and p=0.006 in study II).
Discussion

But the number of teeth was not related to CAD in the multivariate analysis (I).

In study I, subjects had higher numbers of preserved teeth compared to findings in cross-sectional studies that reported an association with CAD (Morrison et al., 1999; Elter et al., 2004; Tu et al., 2007).

Holmlund et al. (2010) investigated whether or not oral parameters were associated with future CVD death (median follow-up time of 12 years). Their study included subjects with periodontal disease and random subjects from the general population (ages 20–89), which is a wider range than study I.

The dose-dependent association between number of teeth and overall death and CAD-related death reported by Holmlund et al. (2010) remained significant after adjustment for age, gender, and smoking, although no association was found in terms of periodontal variables. Loss of teeth might be an effect of periodontitis, but tooth loss can obviously occur for other reasons, e.g., caries and related complications. A low number of teeth on the individual level might be due to socioeconomic and lifestyle factors or perhaps subjects do not have equal preventive dental care opportunities.

The main purpose of study II was to investigate CAD-related morbidity and mortality in terms of myocardial infarction, revascularisation treatments or CAD-related death (CAD endpoints) and periodontal disease. Results from study II did not show significant association in the CAD patient group with baseline periodontal status and CAD-related endpoints, which is in contrast to results reported by Renvert et al. (2010). The finding in our study might reflect a result from successful treatment of CAD and CAD prevention and an improved periodontal health.

In study II, death due to CAD was confirmed from data in Sweden’s death index. MI, PCI, and CABG events that afflicted the re-examined subjects were recorded from medical records from the cardiac clinics. Out of the baseline sample of 161 CAD patients and 162 control subjects, 126 CAD patients and 121 control subjects were periodontally re-examined. These are fairly high proportions considering the follow-up occurred eight years after baseline and a subject could be up to age 75 at inclusion. The overall number of deceased subjects at the time for re-examination was 35 (mean age at death was 66.7 years, range 51–75). Interestingly, 30 out of the 35 deceased subjects showed periodontitis at baseline: 23 (66%) had moderately advanced periodontitis and 7 (20%) had severe periodontitis.

Subjects, who withdrew from study II, did not attend re-examinations for a range of understandable reasons, e.g., work, travel, or illness not related to
Discussion

CAD. Even so, the withdrawals could have influenced results. With a larger sample size or a longer follow-up period, results might have been different.

Interestingly, a tendency was found in CAD-related endpoints and periodontal disease in the total baseline sample of 323 subjects (161 CAD patients and 162 control subjects \(p=0.052\)). I.e., quite many subjects with severe periodontitis (groups 4 and 5) were afflicted by a CAD-related endpoint, compared to subjects who had moderate or no periodontitis. This might indicate that periodontal disease might be used as a predictor for CAD in the future. In Sweden, a high proportion of the population regularly visits a dentist, so one could speculate that diagnosis of periodontitis might be used as a marker for increased CAD risk. If future studies definitely prove that periodontitis is a true risk factor for CAD, the periodontitis treatment may be a part in CAD prevention.

Studies have reported a CAD-periodontitis association (Bahekar et al., 2007) but understanding of the mechanisms behind it is only in a nascent phase. We might speculate on ways in which periodontitis might trigger CAD. Inflammatory response to subgingival microbiota is a defence and wound-healing mechanism. In periodontitis patients, these responses vary, compared to subjects without periodontitis, because tissue destruction exceeds regeneration (Kinane et al., 2011). CAD pathogenesis is complex and in the worst cases, inflammatory processes cause ruptures of the fibrofatty plaques or superficial erosion in the walls of the coronary arteries (Libby, 2008).

Studies III and IV studied HGF, a multifunctional healing factor involved in repair and regeneration of various tissues and organs (Nakamura et al., 2011). HGF is also associated with CAD (Soeki et al., 2000). CAD patients (III) showed higher serum concentrations of HGF compared to age- and gender-matched healthy blood donors \(p<0.001\), which is aligned with other studies (Watanabe et al., 2001). High concentration of HGF is known to reflect chronic inflammation (Funakoshi and Nakamura, 2003) but the HGF must be biologically active to induce healing. HGF is produced as an inactive single-chain molecule that is subsequently converted into a two-chain, biologically active heterodimer (Mars et al., 1993). This is most essential because only the biologically activated HGF induces, e.g., mitogenesis and motility of the target cells. In addition, the biological activity of HGF depends on HGF interaction with its c-MET receptor, through binding to the heparan sulphate proteoglycan (HSPG) co-receptor (Rubin et al., 2001). The c-MET receptor is expressed on epithelial, endothelial, and mesenchymal cells (Nakamura et al., 2011), and HSPG is present on the cell surface in essentially all tissues and in
the extracellular matrix (Nakamura et al., 1986). So analysing the binding affinity to HSPG and c-MET reflects the biological activity of HGF.

In study III, the binding affinity to HSPG (one month after PCI) increased in CAD patients without alveolar bone loss but was unchanged in CAD patients with periodontitis (moderately advanced or severe). This indicates that CAD patients with periodontitis had high concentration of HGF but that the biological activity as a response to PCI was lower. This might indicate that in chronic inflammatory conditions (here, periodontitis), the binding affinity to HSPG is decreased, possibly via proteolytic cleavage and thus inactivation of HGF. When the interaction with the receptor is disabled, then this causes elevated circulatory HGF levels (Liu et al., 1997). The PCI performed in CAD patients (III) might temporarily open the occluded vessel and improve the blood flow but might not change the inflammatory process of atherosclerosis in the vessel wall. These observations support the role of chronic inflammation in CAD pathophysiology.

Since HGF concentrations were higher – but the biological activity tended to be lower in patients with CAD and periodontitis compared to CAD patients without periodontitis – we studied the effect on HGF from periodontitis per se, in otherwise medically healthy subjects (study IV). The concentrations of HGF were significantly higher in saliva (p<0.001), gingival crevicular fluid (GCF) (p<0.0001), and serum (p<0.001) in periodontal patients compared to control subjects without periodontitis. The high concentrations of HGF in saliva and gingival crevicular fluid are aligned with results from other studies (Rudrakshi et al., 2011). The high serum concentrations of HGF might reflect the systemically inflammatory impact from periodontitis. Subjects in study III were included only if having no of the diseases and medications in the exclusion criteria i.e. diabetes mellitus or rheumatoid arthritis, and this support the observed differences.

Although the concentration of HGF was increased in periodontitis patients, the binding affinity to HSPG and c-MET in saliva (p<0.0001) and GCF samples (p<0.0001 for HSPG and p<0.01 for c-MET) was reduced (IV). This ought to negatively influence wound healing and regeneration, at least locally in the periodontal tissues but may also have a systemic effect. Because healing is essential in periodontitis patients, one might expect that periodontitis patients should have higher biological activity of HGF in serum. Interestingly, no significant differences were found in the binding affinity of HSPG between subjects with or without periodontitis, although a difference between patients and controls in the c-MET binding (as a response to subgingival debridement) was measured 3 minutes after debridation (p<0.05). This indicates an instant
systemic response to this treatment. Future research needs to find out if periodontitis treatment has an impact on the biological activity of HGF in terms of increasing the activity and thereby improving wound healing and tissue regeneration. This might be of importance in the CAD-periodontitis association.

Periodontitis causes a systemic inflammation, in terms of inflammatory increased serum levels of inflammatory factors such as CRP, II-1, II-6, TNF-γ etc. (Tonetti, 2009) as a response to subgingival micorbiota. This might have an impact on CAD-related inflammatory processes. High HGF levels in serum (III, IV) might be an effect from locally produced HGF and/or produced systemically, because bacteria and bacterial products associated with periodontitis induce inflammatory mediators (Teles and Wang, 2011). Periodontal patients (IV) had higher prevalence of periodontitis-associated microbes than periodontally healthy control subjects. This was not unexpected because the patient group consisted of subjects with untreated severe periodontitis with deep periodontal pockets. Subgingival plaque samples in our study were collected from the deepest pocket with bleeding on probing in each quadrant and the samples were pooled. We had expected higher proportions of some of the analysed species. P. gingivalis was found in 53% of the periodontal patients. This is equal to the prevalence reported for P. gingivalis on site level (Preus et al., 1995).

Presence of P. gingivalis in the subgingival samples in study III was analysed using PCR (Slots et al., 1995) – a technology with high sensitivity. In study IV, prevalence of 12 periodontal pathogens and 6 complementary species were analysed using checkerboard DNA-DNA hybridisation technology (Socransky et al., 1994), a method that uses DNA probes for multiple species. This method is reported to give high sensitivity but the specificity might be impaired due to cross-reactions between species (Socransky et al., 2004). The selected methods may detect prevalence of included species and thus the found prevalence might be correct, although the results are confusing.

No differences in serum HGF concentration were found in CAD patients with or without P. gingivalis (study III). Nor did the concentration or the binding affinity to c-MET and HSPG differ according to prevalence of the 12 studied periodontal pathogens in subjects with or without periodontitis (study IV). Uehara et al. (2005) proposed P. gingivalis gingipain to stimulate HGF production from gingival fibroblasts but in our studies, P. gingivalis did not differentiate HGF concentrations found in saliva and GCF (study IV) or in serum (studies III, IV). Other factors from periodontitis or other species and/or complexes of species not studied might have a higher impact on HGF
concentrations and biological activity than *P. gingivalis*. In larger study populations, effects from *P. gingivalis* or other species or complexes of species, may also be more distinct.

In all our studies, tobacco use is a confounding factor that should be considered. Smoking habits (including previous smoking) were more prevalent among CAD patients (I, II) and among periodontal patients (IV) compared to subjects in the control groups, and this might influence the results. HGF concentrations have been positively associated with smoking (Yamamoto et al., 2001; Lieb et al., 2009), but the impact from smoking on the biological activity of HGF remains unclear. Other factors reported to be associated with HGF concentrations are age, female sex, diastolic BP, antihypertensive treatment, diabetes, triglycerides, BMI, and an inverse association with HDL cholesterol (Lieb et al., 2009). Subjects with diabetes were excluded from the study populations (I-IV). In study III, higher numbers of CAD patients were male. In studies I, II and IV, the included subjects were matched for age and gender and thus influence from these factors ought to have had lower impact. Factors not analysed in our studies, such as BMI and serum cholesterol levels, might have had an impact on the results. CAD patients (I, II, III) were almost all put on statins as a routine treatment before PCI, but it would have been most valuable if serum cholesterol levels had been measured before the start of this medication.

Another shortcoming in study I and II was that general health status in control subjects was self-reported. To confirm no CAD in the control group, it would have been preferable to do coronary angiography, but this could not be done for practical and ethical reasons. Analysing inflammatory mediators in serum samples from CAD patients and controls in study I would have been most valuable, but the study was designed in 2000, when knowledge of the CAD-periodontitis association was more limited.

The results reported in this thesis support a CAD-periodontitis association. CAD patients demonstrated significantly higher periodontal disease prevalence and severity than individuals without CAD, but there was no significant association between periodontal disease-CAD outcomes during the eight-year follow-up. The impact from periodontitis on HGF and its HSPG and c-MET receptors, and the effect of periodontal-associated bacteria on biological activity of HGF and the proposed effect on CAD must be further studied. Nevertheless, our results may have an impact on future strategies when it comes to CAD diagnosis, evaluation, prevention, and treatment. Studies that strive to acquire more knowledge about CAD – one of the major causes of disease and premature death in the industrial world – are very
important, because known risk factors cannot explain all cases and thus knowledge of the potential role that periodontitis plays is crucial. Just like other inflammation-related diseases, periodontal disease should be considered a factor in CAD development. The oral cavity is an important part of the human body, and oral health should be accounted for in CAD research. The importance of healthy periodontal tissues and effects from periodontal treatment on systemic inflammation and its effect on CAD must be further studied.
CONCLUSIONS

Main conclusions from the studies were:

- CAD patients appear more often to have severe periodontitis when it comes to deep pockets, bleeding after pocket probing, and on radiographs, overall alveolar bone loss that exceeds one-third of the root length – compared to subjects without CAD. Subjects without CAD-related complaints were more often periodontally healthy (I).

- In the long run, periodontitis is more prevalent in CAD patients. No differences in CAD endpoints in relation to baseline periodontal status, was observed among the CAD patients during the eight-year follow-up (II).

- CAD patients with periodontitis had higher HGF serum concentrations compared to CAD patients without periodontitis. A tendency was found for lower HGF biological activity in CAD patients with periodontitis, which indicates that periodontitis has a systemic effect (III).

- Healthy subjects with severe periodontitis had higher HGF concentrations in serum, saliva, and gingival crevicular fluid, compared to healthy subjects without periodontitis, which indicates that periodontitis triggers local and systemic effects. HGF biological activity was decreased in saliva and gingival crevicular fluid in subjects with periodontitis (IV).
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