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ORAL BACTERIA MICROBIOME IN CAROTID ATHEROSCLEROTIC PLAQUE

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Experience is the name everyone gives to their mistakes

Oscar Wilde

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1.INTRODUCTION

1.1 ATHEROSCLEROSIS

The word atherosclerosis is derived from the Greek meaning both softening (athere) and hardening (skleros) and refers to a complex disease process affecting the major blood vessels of the body. It is a disease that has plagued humans for centuries.

1.1.1 HISTORY

It was not until the mid-seventeenth century that a process that resulted in degeneration of the arteries with advancing age was recognized. In 1755, the Swiss physiologist Albrecht von Haller reported on progressive atherosclerotic changes in the blood vessels of the elderly[1]. Later, in 1761, the Italian physician and pathologist Giovanni Battista Morgagni heralded the idea of using microscopic evaluation of tissues to correlate disease with histology. His work, and that of his pupil Antonio Scarpa, correlated a lesion that they described as similar to an ulcerated plaque to aneurysm formation[2]. Thus, atheromatous lesions became the focus of study—first, as a precursor to aneurysm formation, and then, as a separate pathologic entity. Later, a surgeon, Joseph Hodgson, in London proposed that inflammation was the underlying cause of these plaque formations and hypothesized that the process was linked to the intimal layer of blood vessels. Rokitansky was one of the first to observe and document that there were both thrombogenic and calcific components in atherosclerotic lesions[3]. Rudolf Virchow concluded that atherosclerotic lesions were located in the intimal layer and described the process of plaque formation that was initiated by the formation of a coagulum which he called thrombus. The studies of Alexander Ignatovski and Nikolai Anitschkovin the early 1900s demonstrated that atherosclerotic changes could be induced in animals by a diet rich in cholesterol[4]. This led to the important discovery in 1910 by German chemist Adolf Windaus that human atherosclerotic lesions contained cholesterol.

HISTORICAL EVOLUTION OF THE UNDERSTANDING OF VASCULAR DISEASE

NAME	YEAR	CONTRIBUTION
Andreas Vesalius and Gabriel Fallappio	1550s	Described aortic and peripheral aneurysms
William Harvey	1628	Described cardiovascular system as a circuit

NAME	YEAR	CONTRIBUTION
Daniel Sennet	1628	Described arteries as comprised of two concentric layers
Albrecht von Haller	1755	Described progressive changes within arterial walls
Giovanni Battista Morgagni	1761	Described microscopic changes occurring within atheromas
Antonio Scarpa	1804	Correlated ulcerated atheromatous lesions with aneurysmal development
Joseph Hodgson	1815	Proposed inflammation as a cause of atherosclerosis
Jean Lobstein	1829	Coined the term arteriosclerosis
Carl Rokitanski	1852	Detailed descriptions of early and mature atheromatous plaques
Rudolf Virchow	1854	Described the process of thrombosis and embolism
Alexander Ignatovski	1908	Experimentally induced atherosclerosis in rabbits
Adolf Windaus	1910	Discovered cholesterol within atherosclerotic lesions
Nikolai Anitschkov and Ludwig Aschoff	1933	Provided summaries of early experimentation and results regarding the research of atherosclerosis

1.1.2 EPIDEMIOLOGY

Atherosclerosis is the principal cause of heart attack, stroke, and peripheral vascular disease and remains a major contributor to morbidity and mortality in the Western World.

Cerebrovascular disease is also a major consequence of the atherosclerotic process. Stroke, with an incidence of 500,000 cases yearly, is the third leading cause of death in the US. In one study, the annual stroke rate was determined to be 1.3% per year in patients with up to 75% carotid stenosis and the rate of stroke is nearly tripled in patients with higher-grade lesions[5]. Thus, the results of untreated or poorly treated atherosclerotic disease has significant medical consequences.

1.1.3 ETIOPATHOGENESIS

Disease progression is slow, beginning in childhood and usually becoming clinically manifest in middle age or later. Although the aetiology of atherosclerosis is not fully understood, it is generally accepted that atherosclerosis is a multifactorial disease induced by the effects of various risk factors on appropriate genetic backgrounds. Many risk factors, such as hypercholesterolemia, modified lipoproteins, hypertension, diabetes, infections and smoking have been identified in the development of atherosclerosis.

Atherosclerosis has been the focus of intense research for over 100 years. Since Anitschkow and Chalatow first reported that cholesterol can cause atherosclerosis, many investigators have

intensively studied the role of blood cholesterol in the pathogenesis of atherosclerosis. New insights into the pathogenesis of atherosclerosis have emerged during the last decades, due to the progress of cellular and molecular approaches to the study of cell interactions in the arterial wall as well as alterations of lipid metabolism. More recently, a widely accepted hypothesis is that atherosclerosis is an inflammatory disease, because recent advances in the basic science have established a fundamental role for inflammation in mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis[6].

In summary, atherosclerotic vascular disease is a chronic inflammatory disease of arteries characterized by the invasion, proliferation and accumulation of cells from the arterial media (smooth muscle cells) and the circulating blood (monocytes/macrophages and T lymphocytes) in the intimal layer, with deposition of connettive tissue and lipids. This disease is characterized by accumulation of low-density lipoprotein in the arterial intima, expression of leukocyte adhesion molecules and chemokines by activated endothelium, promoting the recruitment of innate and adaptive immunity cells (monocytes and T cells) to the intima, thus resulting in local inflammation process. Inflammation and increased oxidative stress play important roles in the pathogenesis of atherosclerosis and plaque instability[7-9]. Therefore, human atherosclerosis can be considered both a metabolic and an inflammatory disease.

1.1.4 ARTERIAL WALL AND PLAQUE DEVELOPMENT

The intima of large and medium sized arteries is composed of a monolayer of endothelial cells and matrix proteins and occasional smooth muscle cells in the subendothelial space. The media of the vessel contains smooth muscle cells and the elastic lamina built by matrix proteins, while the main component of adventitia is connective tissue. With increasing age, the diseased arterial wall slowly thickens and develops focal lesions of lipid accumulation in the intima. These early lesions are known as fatty streaks and are thought to be the sites of predisposition to advanced lesions called atherosclerotic plaques or atheroma, which may lead to clinical symptoms in certain circumstances. The atherosclerotic plaque or atheroma is the advanced lesion and consist of a dense accumulation of extracellular lipid, known as the lipid core, occupies an extensive but well-defined region of the intima[10]. No increase in fibrous tissue and complications such as defects of the lesion surface and thrombosis are present at this initial stage of disease. The characteristic core appears to develop from an expansion and confluence

of the small isolated pools of extracellular lipid that characterize atheroma. Between the lipid core and the endothelial surface, the intima contains macrophages, smooth muscle cells, lymphocytes and mast cells. Capillaries surround the lipid core, particularly at the lateral margins and facing the lumen. Frequently macrophages, macrophage foam cells, and lymphocytes are more densely concentrated in the lesion periphery. Much of the tissue between the core and the surface endothelium corresponds to the proteoglycan-rich layer of the intima, although infiltrated with the cells just described. Advanced lesions may or may not narrow the arterial lumen, nor be visible by angiography, nor produce clinical manifestations. Such lesions may be clinically significant even though the arterial lumen is not narrowed, because complications may develop suddenly[10].

In addition, two types of atherosclerotic plaques, i.e. ‘vulnerable’ and ‘stable’ plaques, have been recognized[8]. Vulnerable plaques often have a well-preserved lumen, since plaques remodel outward initially. The vulnerable plaque typically has a substantial lipid core and a thin fibrous cap separating the thrombogenic macrophages bearing tissue factor from the blood. Clinical data suggest that stable plaques more often show luminal narrowing detectable by angiography than do vulnerable plaques, but with much less chance of rupture.

1.1.5 RISK FACTORS

Accumulating evidence suggests a causal relationship between blood cholesterol and atherosclerosis. It is established that familial hypercholesterolemia related to increased LDL levels causes premature atherosclerosis and heart disease[11], whereas non-genetic hypercholesterolemia is also associated with the development of atherosclerosis.

Hypertension is another well-established risk factor for atherosclerosis[12]. Clinical trials have shown that, in the highest quintile for diastolic pressure, hypertension still contributes significantly to the risk of atherosclerosis, even with the added risks of high cholesterol and smoking. The fact that atherosclerotic lesions preferentially occur in the areas where hemodynamic or biomechanical stress is altered, e.g. bifurcation of the arteries, supports the idea that hypertension exerts its role in the pathogenesis of atherosclerosis via altered mechanical stress to the vessel wall. Although veins do not develop spontaneous atherosclerosis-like lesions, accelerated atherosclerosis occurs rapidly in venous bypass grafts,

which bear increased biomechanical forces due to alterations in blood pressure, i.e. vein (0-30 mm Hg) *vs.* artery (120 mm Hg). This finding supports the hypothesis that mechanical stress could be a crucial factor in the pathogenesis of atherosclerosis.

As mentioned, atherosclerosis is a highly prevalent disease in humans with significant morbidity and mortality[13] and is one of the leading cause of death and one third of population have some form of the disease, which includes coronary disease, cerebrovascular disease and peripheral artery disease[14]. However, half of those with the disease do not have traditional cardiovascular risk factors such as obesity, hypercholesterolemia, hypertension, history of smoking or genetic background[15], and thus the cause(s) of rapid atherosclerotic plaque progression and disease is unknown in many patients. Traditional risk factors only explain a proportion of the incident cases of all atherosclerosis. There is a body of evidence that microorganisms play a role in the pathogenesis of atherosclerosis and may be a primary risk factor in people who do not suffer from other established risk factors.

1.1.6 INFECTIOUS HYPOTHESIS

The infectious hypothesis of atherosclerosis has been studied for some decades. Assuming involvement of both innate and adaptive immune systems in atherogenesis, infections with bacteria and/or viruses have been supposed to potentially contribute to the pathogenesis of atherosclerosis via direct and indirect mechanisms.

Infectious agents represents a major source of systemic inflammatory response activation with the potential to accelerate plaque growth and instability[16]. There is a substantial evidence demonstrating an association between the induction of inflammatory responses induced by infectious agents and the acceleration of atherosclerosis[14, 16-19] and it has been postulated that chronic infections can contribute to the development of atheroma by direct (platelet aggregation, invasion of endothelial cells and endothelial injury) and indirect (induction of intracellular adhesion molecules, production of antibodies to lipopolysaccharide as well as cytokines and disfunction of the immune system) mechanisms[20]. Several microorganisms including Chlamidia Pneumoniae and cytomegalovirus have been reported to be implicated in the infectious etiology of atherosclerosis[21]. However, the data are inconsistent, with other studies showing no increased risk for atherosclerosis[22, 23]. One possible explanation for this

disparity is that infections contributing to atherosclerosis risk may depend, at least in part, on the host's response to the pathogen, i.e. inflammatory and immune reactions.

Among the various infectious agents, periodontal pathogens are prominent contenders in the infectious hypothesis because of the chronic inflammation associated with periodontal disease. There is also a significantly increased prevalence and incidence of coronary artery disease indicating that periodontal disease predicts coronary artery disease[24]. Although there are over 500 bacterial species in the oral cavity, probably only a few species are implicated in the progression of periodontal disease.

1.2 PERIODONTAL DISEASES

Periodontal diseases are complex multifactorial diseases caused by polymicrobial subgingival biofilm with immune and inflammatory responses. The polymicrobial nature of periodontal disease promotes chronic inflammation and orchestrates a complex disease mechanism in which inflammation results in the destruction of the periodontium (alveolar bone, cementum, periodontal ligament and gingiva). It has been recently revealed that induction of periodontitis is actually more complex and involves the entire microbial community present in the oral cavity. Periodontal inflammation is thought to be largely mediated by 'pathobionts' which are commensal organisms that under conditions of disrupted homeostasis (i.e. during P. gingivalis infection) have the potential to deregulate the inflammatory response and cause disease[25].

1.2.1 PERIODONTAL AND CARDIOVASCULAR DISEASE RELATION

Given the high incidence of periodontal and cardiovascular diseases and their economic cost to society, defining their mechanistic link has become increasingly important. This is illustrated by the increasing number of reports published on the link between periodontal disease and cardiovascular disease in recent years; in 2007, only 73 articles were cited on this topic; however, by 2014 there were close to 4000[26].

The story begins in 1989 when two Scandinavian reports revived a century-old hypothesis relating chronic infections with vascular disease that originally was proposed by French and German scientist[27, 28]. Mattila and colleagues[29] found high markers of oral infections more frequently in patients with recent myocardial infarction than in health control patients. Syrjanen and colleagues[30] observed relatively poor oral health among patients who had experienced a recent stroke compared with control patients who had not experienced stroke. These authors

drew careful conclusions, primarily because of the substantial overlap noted between risk factors for both periodontal disease and cardiovascular disease: being older, being male, cigarette smoking, diabetes and low socioeconomic status. If periodontal disease and cardiovascular disease simply share common risk factors a correlation between the two would be expected even if a causal link did not exist. The geographical homogeneity and small number of patients enrolled in these early studies precluded any reliable generalizations beyond the specific study population. Subsequently, studies addressing many of these limitations have made substantial contributions to clarify the association[31, 32]. Many studies reported positive associations after accounting for the effects of multiple risk factors such as age, sex, diabetes, cholesterol levels, blood pressure, obesity, smoking status, dietary patterns, race, education and socioeconomic status[33-37]. These results have particular importance in the case of smoking but there is the possibility of an health bias effect, in which people who smoke are more likely to have unhealthy lifestyles, which can lead to both periodontal and cardiovascular diseases. Grau and colleagues[38] provide important information concerning the specificity of the hypothesis to periodontal disease; they reported a 400 percent increase in stroke risk associated with periodontitis but found no relationship between caries and strokes. These findings argues against a healthy lifestyle bias. Not all studies have found a positive relationship between periodontal and cardiovascular disease. Reports from the Health Professional Follow-Up Study[39] and the Physicians' Health Study[40] observed no association between periodontal disease and either coronary artery disease or stoke among more than 66000 male health professionals. The large sample sizes provide a good reason for caution but a major limitation of these studies stems for the self-reported nature of periodontal disease assessment.

Interestingly studies reporting no association between periodontal disease and coronary disease are at odds with stroke findings from the same population. These discrepancies are consistent with the literature, which indicates that periodontal disease might be a stronger risk factor for cerebrovascular disease than for coronary disease[28, 31].

Reports from the United States[37] and Germany[38, 39] have provided evidence that the association between periodontal disease and cardiovascular disease might be stronger among men than among women. The possibility that novel risk factors might partly explain some of the sex differential in cardiovascular disease is intriguing[41].

In summary, since 2003, research supports a moderate relative association between cardiovascular disease and periodontal disease[31, 32]. This relationship appears to be more pronounced in younger participants, possibly different in male and female subjects and consistently stronger for clinical stroke outcomes. Nevertheless, substantial variation in results among studies is apparent and other research designed to measure oral infection exposure and mechanisms that link with atherosclerosis were needed[28].

During the period between 2003 and 2006, four separate large clinical trials were designed to treat coronary artery disease patients with antibiotics to alleviate disease progression, although these agents were not specific for periodontal pathogens[42-45]. The results from these trials, each of which enrolled over 4000 patients with coronary artery disease, showed no significant long-term benefit of antibiotic treatment in those with established disease. Following the publication of the results of these separate studies, interest in further examining the link between infection and cardiovascular disease considerably declined.

As already mentioned the study of periodontal disease and cardiovascular disease association has its roots in the broader hypothesis concerning infections and cardiovascular disease. Therefore recent research has provided new insights by obtaining more precise measures of exposure to periodontal microbes.

Many studies demonstrated that periodontal disease-associated bacteria enter the blood stream during mastication, brushing and flossing teeth and during dental procedures[46]. Dental plaque has been considered as the reservoir of the most pathogenic bacteria and the past evidence states that dental plaque is the primarily etiological factor for the progression of periodontal disease[21].

The rationale is that patients with periodontitis are chronically exposed to non symptomatic bacteriemias at increased levels, for longer duration, and with greater microbial diversity of infections, which increase with periodontal disease severity[47]. Some studies also reported that elevated antibodies to selected periodontal pathogens titers were associated with increased atherosclerosis even among never-smokers[48-52].

A direct approach to assessing exposure to periodontal microbes is to measure bacteria quantitatively in periodontal and atherosclerotic plaque samples.

Several studies have attempted to demonstrate the presence of periodontal bacteria or their components in human atherosclerotic lesions. A distinct pathogenic consortium of

Porphyromonas Gingivalis, Treponema Denticola and Tannerella forsythia (the red complex) is found in subgingival plaque in severe periodontitis and bacterial genomic 16S rDNA from numerous oral and periodontal species, including Porphyromonas Gingivalis, Treponema Denticola, T. Forsythia and F. Nucleatum have been detected in human clinical atherosclerotic plaque lesions[17, 53-55]. Chiu [56] performed a histologic analysis of human endoatherectomy carotid specimens and found P. Gingivalis and Streptococcus Sanguis in unstable plaques. Ishihara et al[57] detected Actinobacilus Actinomycetemcomitans, Bacteriodes Forythus, Treponema Denticola and Campylobacter Rectus in samples of coronary artery plaques by polymerase chain reaction. Furthermore, the presence of A. Actinomycetemcomitans, Fusobacterium nucleatum-periodonticum-simiae group, P. Gingivalis, Prevotella Intermedia, Prevotella nigrescens and T. Forsythia in atheromatous plaques from coronary arteries have been demonstrated by Gaetti-Jardim Jr et al[58] using PCR.

Then in recent years, many researchers have focused their attention on the ability of periodontal pathogens to colonize in the atheromatous plaque in order to substantiate the potential role of the periodontal bacteria in the progression of atherosclerosis. Nevertheless, a clear correlation between the detection rates of periodontopathic bacterial DNA in atheroma and periodontal pockets has not been established. An indian study from Mahindra et al[21] revealed the presence of the red complex bacteria (P Gingivalis, T. Denticola, T Forsythia) in coronary atherosclerotic plaque in patients underwent CABG. Takahiro et al[59] detected DNA of periodontal bacteria in coronary thrombi in 22.2% of patients associated with myocardial infarction. But with current knowledge, we cannot answer the question of whether periodontal bacteria become attached to already existing atherosclerotic lesions or these bacteria promote the atherosclerosis and induce instability of plaque.

1.2.2 MECHANISMS BY WHICH PERIODONTITIS MAY RELATE TO CARDIOVASCULAR DISEASE

A number of reviews have been published that outline potential biological mechanisms linking infections and periodontal disease to cardiovascular disease[60-62].

1.2.2.1 DIRECT PATHWAYS

Oral microbes and their byproducts can gain systemic access via the circulatory system. Gentle mastication can induce endotoxemia and this risk is elevated according to an increased severity

of periodontal disease[63], also dental procedures and toothbrushing can induce bacteriemias[46, 64]. It is this common and recurrent transient bacteremia that has been proposed to produce chronic insult to the vasculature and contribute to the injury and inflammation that initiates the development of atherosclerosis[25].

In gaining, systemic access, oral microbes have the potential to directly influence subclinical mediators of cardiovascular events such as hypercoagulability, atherosclerotic development or both. Animal studies demonstrated that intravenous inoculation with P Gingivalis accelerates atherosclerotic developement[65] and oral inoculation of P Gingivalis demonstrate accelerated early atherosclerosis with finding of bacterial DNA in the aortic tissue[18]. In vitro studies demonstrate the ability of Streptococcus Sanguis and P gingival to induce platelet aggregation and hypercoagulability[28, 62, 66].

1.2.2.2 INDIRECT PATHWAYS

Atherosclerosis, as mentioned, has a strong inflammatory component, and epidemiological evidence suggests that increased levels of systemic inflammation are predictive of cardiovascular events[67]. People with periodontal disease have elevated levels of systemic inflammatory markers, such as C-Reactive Protein[68]. There are many potential triggers for this enhanced systemic inflammatory response, including transient bacteremias and local release of bacterial byproducts such as lipopolysaccharide[69].

Another plausible mechanism is molecular mimicry, in which antibodies targeted toward bacterial species inadvertently cross-react with host cells[28]. Immune recognition of periodontal pathogens results in progressive inflammation consisting of an immune cell infiltrate, such as monocytes and B and T cells, and production of inflammatory mediators. As inflammation progresses to a more chronic state, the lesion becomes composed of a cellular infiltrate that is predominantly monocytic. Monocytes become activated macrophages, which further accelerate bone resorption through differentiation into osteoclasts and production of tissue-damaging proinflammatory cytokines[25]. Furthermore, additional proinflammatory mediators such as IL-8 and IL-1β have been detected in the gingiva from patients with periodontal disease.

Atherosclerosis begins with a dysfunctional endothelium, resulting in recruitment of a number of immune cells, such as macrophages and T cells, into the lesion. Inflammation within the lesion progresses when immune cells become activated by ligands present in the vasculature,

resulting in the production of a number of proinflammatory mediators that further propagate inflammation[25].

Amongst the most extensively studied markers of inflammation, which have become increasingly associated with inflammatory diseases such as atherosclerosis, are the Toll-like receptors (TLRs)[70]. Receptors in the TLR family recognize specific, conserved pathogen-associated molecular patterns (PAMPs) and, upon ligand recognition, TLRs orchestrate an inflammatory cascade. But it is also known that TLRs can also be engaged by endogenous ligands. Further, endogenous ligands, such as oxidized LDL, have been found to engage TLRs expressed on monocytes cells, resulting in lipid-laden foam cells that characterize the earliest stages of lesion development[71]. This finding, amongst others, gave rise to the notion that ligands of microbial origin may also contribute to the detrimental inflammatory reactions within atherosclerotic lesions. Due to the predominant role of TLRs in atherosclerosis progression, Slocum and colleagues[25] have investigated the association between TLRs and P. gingivalis-mediated atherosclerosis and demonstrated that P. gingivalis-mediated TLR2 activation contributes to atherosclerosis progression. Activation of Toll-like receptor-2 (TLR2) abundantly expressed by endothelial cells of atherosclerotic plaques contributes to endothelial apoptosis and denudation. TLR2 ligands also include some components of gram-positive bacteria, indicating that some infectious factors may function to contribute to atherothrombosis via such mechanism. These data indicate that infectious agents may be at least partially implicated in both pathogenesis of atherosclerosis and manifestation of cardiovascular disease proving that involvement of microorganisms in the processes described above requires additional studies.

Heat shock proteins can also have an important role. Infections with proatherogenic organisms may be important in individuals lacking additional risk factors as well as acting synergistically with established risk factors. In this process, Heat shock proteins may be a link between infections and the pathogenesis of atherosclerosis. Infectious agents may exert their role by producing their own Heat Shock Proteins and inducing host production which could be released into blood. The soluble form of Heat shock Proteins contact endothelial cells and immune cells where innate immune responses are initiated. Innate immune reactions to Heat Shock Proteins result in proinflammatory responses in the vessel wall. Together, infections via Heat shock Proteins contribute to the development of atherosclerosis.

1.3 STROKE AND CAROTID ATHEROSCLEROSIS

Stroke, or cerebral infarction places a significant burden on to patients, their families, the healthcare system and aged-care services. Occlusive disease of the extra-cranial carotid artery is a leading cause of stroke. In 2009–2010 there were 35,345 hospitalisations with a principal diagnosis of stroke and 15,704 for transient ischaemic attack (TIA). Those rates increase substantially with age to be highest in the over 85 year category. The gender distribution was evenly spread with 52% of those hospitalisations for males. Forty-eight percent of hospitalizations were for ischaemic stroke, 29% for haemorrhagic stroke with the remaining 23% unspecified. Approximately 20–30% of ischaemic cerebral infarction result from atherosclerotic disease of either the intracranial or extracranial cerebral blood arteries. This may result from progressive stenosis causing an area of watershed ischaemia, or more commonly turbulence, plaque rupture and embolisation lodging in the terminal cerebral circulation. Around 30% of all cerebral infarctions come from a cardio-embolic source and that proportion may be higher in young people. Carotid artery stenosis or occlusion is thought to be associated with 11.5% of ischaemic stroke (3). The risk of stroke is thought to increase with the severity of stenosis, previous stroke or TIA, age, tobacco use and presence of diabetes. Strokes related to the carotid artery typically affect the brain in the territory of the anterior and middle cerebral arteries.

With this introduction concerning the epidemiology it is clear that management of carotid bifurcation stenosis is a cornerstone of stroke prevention.

Initial hopes that carotid endarterectomy could reverse the clinical course of stroke were proven false, and the role of surgical management of extracranial carotid and vertebral obstructions was defined by one of the earliest efforts at a multicentered randomized clinical trial, The Joint Study on The Extracranial Circulation in 1968[72]. The results of this decade-long study, involving 5000 patients, established the role of carotid endarterectomy in the treatment of minor stroke, transient ischemic attack (TIA), and amaurosis fugax, confirmed that surgery had a limited role in the treatment of established stroke, and established the limited role of vertebral reconstruction in the treatment of cerebral insufficiency. Over the ensuing decades, surgical results of carotid endarterectomy improved, asymptomatic carotid stenosis was increasingly identified by noninvasive studies, and carotid endarterectomy assumed a primarily prophylactic role as prevention of major stroke in asymptomatic patients or those with evidence of transient cerebral

or ocular ischemia. Large randomized trials have established the role and efficacy of carotid endarterectomy (CEA) in stroke prevention[73].

The committee[73] recommends carotid endarterectomy as the first-line treatment for most symptomatic patients with stenosis of 50% to 99% and asymptomatic patients with stenosis of 60% to 99%. The perioperative risk of stroke and death in asymptomatic patients must be <3% to ensure benefit for the patient.

1.3.1 ENDARTERECTOMY

1.3.1.1 HISTORY

Endarterectomy, first performed by J. Cid dos Santos in 1946, was originally designed for simple removal of thrombi. Dos Santos , later, due to bad outcomes, decided to do this operation with the patient under the cover of heparin and stated: "*I really had performed a different operation from the one I originally intended to do; and I could conclude that, under heparin action, blood could flow against muscle without giving place to thrombosis*"

This new procedure, later called thromboendarterectomy, represented a wholly new concept in arterial surgery. It appeared as a revolutionary idea because it seemed to negate the prevailing concept, according to which an injured intima leads inevitably to vascular thrombosis. Indeed, in thromboendarterectomy both the thrombus and the endartery (intima and part of the inner media) are excised.

The accidental finding that arterial thrombosis does not necessarily occur after removal of the intimal lining and a portion of the media is a typical example of "*serendipity*".

Dos Santos's pioneering efforts were soon confirmed and expanded. Originally dos Santos named this operation "arterial disobstruction" or "disobliteration." Later Bazy and Reboul coined the term *endarterectomy*, but Leriche preferred the more comprehensive term *thromboendarterectomy*. These two terms, often used interchangeably, are designed to indicate removal not only of the intima and thrombus but also of the media.

A combination of systemic and local heparinization offers the best method for preventing thrombosis during these usually long procedures. Postoperative heparinization has been abandoned because the possible troublesome complications outweigh its effectiveness in preventing thrombosis at this stage.

1.3.1.2 PATIENT CHARACTERISTICS

The ideal candidate for carotid endarterectomy, would be a normotensive individual without cardiac symptoms, younger than 70 years, preferably male, who had suffered one or more focal cerebral hemispheric transient ischemic neurologic episodes within the preceding 120 days and was found to have 70% to 99% stenosis of the appropriate ipsilateral internal carotid artery at its origin, in the absence of other intracranial or extra cranial arterial lesions on imaging.

The cerebral CT scan ideally should show that the involved cerebral hemisphere received its blood supply via the circle of Willis from the contralateral unaffected internal carotid artery and that the posterior communicating arteries were patent. Scans should not show any sign of cerebral infarction. The patient should have tolerated daily aspirin ingestion, and would be expected to continue to tolerate it for the rest of his life.

Unfortunately, many strokes occur without warning symptoms, and once they have occurred it is usually impossible to reverse the ischemia completely and bring about full functional and anatomic recovery. That stroke can be prevented in symptomatic patients with severe carotid stenoses has been clearly demonstrated[74-76]. The variations in presentation, which may markedly influence surgical risk and long-term outcome, are almost innumerable;

The factors that influence immediate surgical outcome can be classified as:

1) nature and extent of arterial pathology

2) clinical condition of the Patient

3) cerebral pathology

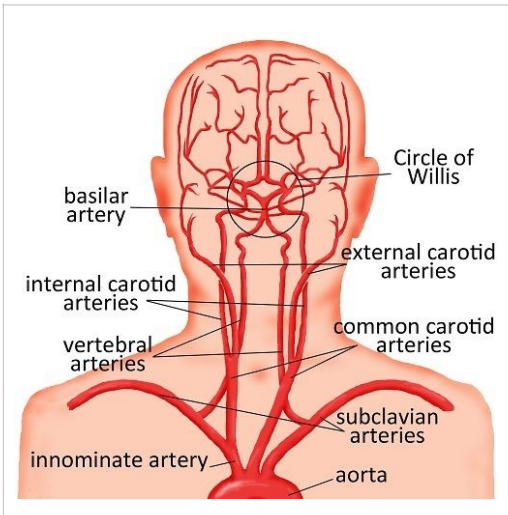
4) experience of the surgeon

The factors that influence late outcome and disease modification can be classified as:

1) specific therapy

2) modification of risk factors

CEREBRAL VASCULARIZATION



1.3.1.3 NATURE AND EXTENT OF CAROTID PATHOLOGY

1.3.1.3.1 The Carotid Plaque

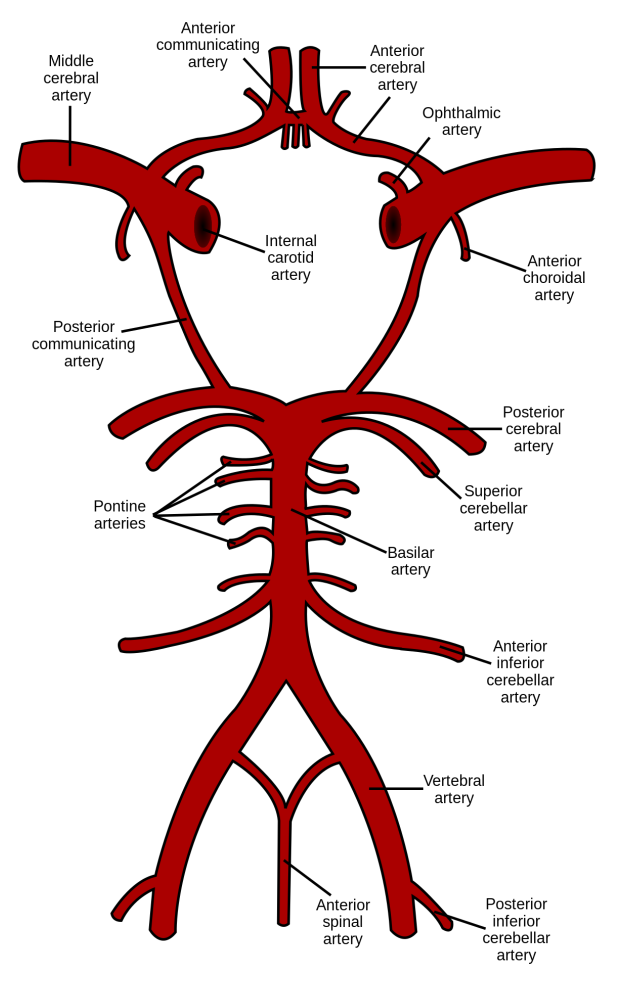
The carotid bifurcation plaque, responsible for approximately 70% of all ischemic strokes, is found in various stages of evolution in both symptomatic and asymptomatic patients[77].

As mentioned, neurologic symptoms and stroke correlate not only with the degree of stenosis, which may impede flow, but also with the nature of the plaque. As the carotid plaque is the result of a sometimes rapidly evolving process and strokes frequently occur without warning, one must conclude that a minimum of 50% to 70% diameter reduction (75% cross-sectional area) in the presence of symptoms characteristic of transient cerebral ischemic attacks, with no other detectable source of microemboli, constitutes an indication for operative intervention if no contraindications exist.

1.3.1.3.2 Unilateral Carotid Involvement

Unilateral and isolated carotid involvement presents the ideal pathologic lesion for surgical intervention because, unless there are major gaps in the circle of Willis, compensatory flow can occur during carotid clamping needed to perform endarterectomy. On occasion, however, even unilateral involvement may be associated with carotid clamping intolerance.

CIRCLE OF WILLIS



1.3.1.3.3 Bilateral Carotid Involvement

When bilateral markedly stenotic plaques are encountered, an order of precedence must be decided based on cerebral angio-imaging and symptoms. Unless one plaque is outstanding because of symptoms threatening early stroke, the concept of carotid predominance has been a useful guide. The carotid artery that supplies the contralateral hemisphere (the "predominant" or "major" artery) is susceptible to clamping intolerance. Therefore the non

predominant or "minor" artery is operated on first, followed by operation one or more weeks later on the predominant artery,

1.2.1.3.4 Stenosis and Occlusion

Stenosis opposite an occlusion requiring operation offers the greatest risks of clamp intolerance and creates the greatest risk of perioperative stroke. Operations in this cohort of patients may incur 10% to 15% operative risk requiring extraordinary measures beyond merely employing shunts routinely. It is essential, as well, to avoid hemodynamic instability, which, even in the presence of a patent intraluminal shunt, may predispose to stroke. Nevertheless, a number of strategies have been used to ensure safety of operations on stenotic carotid arteries opposite occlusions, ranging from operating on conscious patients with selective shunting to the routine use of shunts under general anesthesia.

1.3.2 CLINICAL CONDITION OF THE PATIENT

1.3.2.1 Neurologic Condition

Stenotic arterial disease of the cerebral circulation, which lends itself to carotid endarterectomy, manifests in a variety of ways. Symptoms may occur unexpectedly and suddenly from massive cerebral infarction causing catastrophic nonremediable stroke, or may be evanescent and sometimes difficult to recognize

The severity of the pathologic process in the arteries is not necessarily reflected in the severity of symptoms or in the degree and extent of existing cerebral damage. Frank infarcts may present with minimal or no symptoms as may multiple occlusive or embolizing arterial lesions.

The neurologic status therefore becomes a vital issue in selection of patients for operation, not only to attempt to recognize those who, with minimal or no symptoms, are at risk of suffering catastrophic stroke, but also to estimate operative risks as they relate to clinical status. Of equal importance is whether clinical improvement can be expected to occur in the presence of neurologic impairment.

1.3.2.2 Non-neurologic Factors

1.3.2.2.1 Age

Although age is heavily weighted in evaluating patients for operative procedures, it has become apparent that age itself need not be considered a contraindication to carotid endarterectomy. Therefore, estimation of surgical risks in octogenarians and older individuals should be with reference to specific risk factors other than age.

1.3.2.2.2 Coronary Artery Disease

The concurrence of coronary and carotid artery disease has been frequently convincingly documented as has the impact of one upon the other in the operating room. Even in the absence of clinical symptoms, 14% of patients considered for carotid endarterectomy had angiographic evidence of severe operable coronary artery disease.

Patients with severe coronary artery disease manifested by unstable angina, recent acute myocardial infarction, low left ventricular ejection fractions, markedly positive stress tests, left main coronary stenosis, or "triple vessel disease" require particular consideration for how to manage symptomatic or severely stenotic carotid disease. Strategies range from performing both operations at the same operative session, with neurologic complication rates that range from low to as high as 9.5%, to attempting to "uncouple" the procedures by operating on the more threatening procedure first.

1.3.2.3 Techniques

A successfully performed operation requires strict adherence to a set of principles aimed at avoiding a number of well-defined operative complications, any one of which can destroy the effectiveness of the procedure. The principal complications to be avoided are

1) cerebral clamping ischemia
2) cerebral embolization
3) operative site thrombosis

1.3.2.4 Cerebral Clamping ischemia

The design of the intracerebral circulation, with its circle of Willis, was seemingly to ensure adequate total cerebral blood flow from any one of the extracranial cervical arteries. Its execution, however, has resulted in various gaps in the circle through defects or absences of various communicating arteries that complete the circle, as occurs in 30% of humans even without arterial occlusive disease[1].

When cerebral ischemia occurs, signs may appear within seconds of application of clamps (8 to 30 seconds under local anesthesia), and recovery is equally prompt on their removal or on restoration of flow through temporary inlying shunts. The longest duration of total ischemia compatible with complete recovery in humans is not certainly known but may be as short as 2 to 5 minutes, in any event much too short to permit the completion of a well-performed carotid endarterectomy. The problem is complicated by the fact that unilateral carotid endarterectomy

rarely results in total ischemia because some circulation is usually maintained through collaterals, and only when regional flow is decreased 64% signs of cerebral ischemia appear.

Well-functioning intralumenal shunts are the most reliable devices that correct clamping cerebral ischemia.

1.3.2.5 Operative Site Thrombosis

Operative site thrombosis may occur in the absence of symptoms, may cause only transient ischemic neurologic deficits, or may precipitate catastrophic stroke and death. Mechanisms leading to thrombosis usually result from technical errors such as incomplete removal of plaque, from edges or intimal flaps in the internal carotid artery at the distal termination of the endarterectomy, from uncorrected kinks of the internal carotid artery that become accentuated following endarterectomy, from stenosis produced by primary arteriotomy closure, from posterior wall buckling caused by too short a roof patch for the length of the arteriotomy, from stasis clot forming in the isolated endarterectomized segment during arteriotomy closure, due to inadequate heparinization, insufficient flushing, or from insufficient irrigation of the endarterectomy site just prior to final closure of the arteriotomy.

Reexploration of the operative site is usually indicated upon detection of an early neurologic deficit. Delay beyond *1 or 2* hours may result in permanent neurologic damage.

2. STUDY

2.1 MATHERIALS AND METHODS

Is a prospective monocentric study and the aim is to ascertain the presence of periodontal bacterial DNA in carotid atheromatous plaque in dentate patients and to assess the concomitant presence of the same microbiome, if any, in both periodontal pockets and carotid atheroma in each dentate patient.

2.1.1 PATIENTS SELECTION

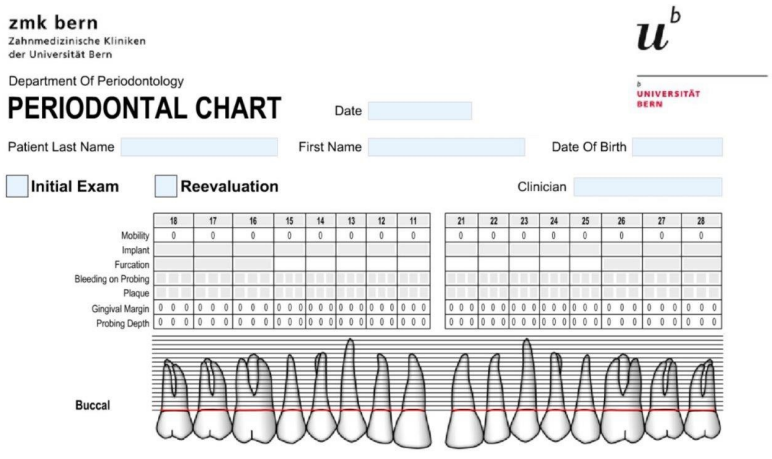
Six patients scheduled to undergo carotid endarterectomy at Centro Cardiologico Monzino were recruited for this study and they were cooperative and readily accepted for the study. The patients were identified as candidate for carotid endarterectomy according to the standard medical practice and guidelines (all patients were diagnosed with atherosclerotic disease involving the internal carotid artery documented by color duplex ultrasound and contrast CT scan with ≥70 % stenosis) .

Inclusion criteria were age > 18 years old, surgical indication (based on international guidelines), signature of informed consent.

Exclusion criteria includes antibiotic intake or an history of periodontal treatment carried out in the previous six months, edentulous subject, chronic inflammatory pathologies that requires steroid or immunosuppressive therapy.

The informed consent was obtained from the subjects. The Ethics Committee of the Centro Cardiologico Monzino/Instituto Europeo di Oncologia approved the protocol of this study.

Medical and dental history of each patient was obtained by an interview. The periodontal examination was done using periodontal chart from ZMK Bern University.


zmk bern
Zahnmedizinische Kliniken
der Universität Bern

Department Of Periodontology

PERIODONTAL CHART Date

Patient Last Name First Name Date Of Birth

Initial Exam Reevaluation Clinician

	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Mobility	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Implant																
Furcation																
Bleeding on Probing																
Plaque																
Gingival Margin	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Probing Depth	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Buccal

zmk bern
Zahnmedizinische Kliniken
der Universität Bern

Department Of Periodontology

PERIODONTAL CHART

Date

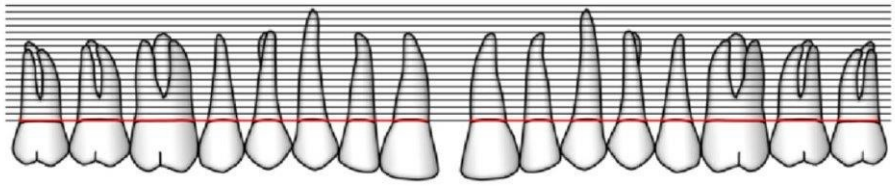
UNIVERSITÄT BERN

Patient Last Name | First Name | Date Of Birth

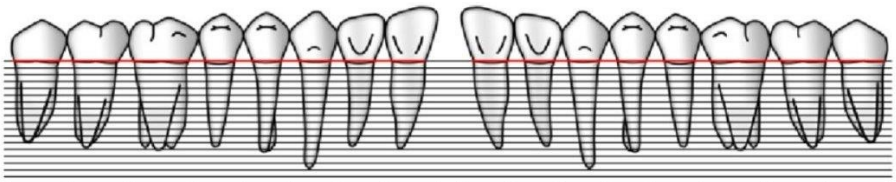
☐ Initial Exam ☐ Reevaluation Clinician

	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Mobility	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Implant																
Furcation																
Bleeding on Probing																
Plaque																
Gingival Margin	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Probing Depth	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Buccal



Lingual

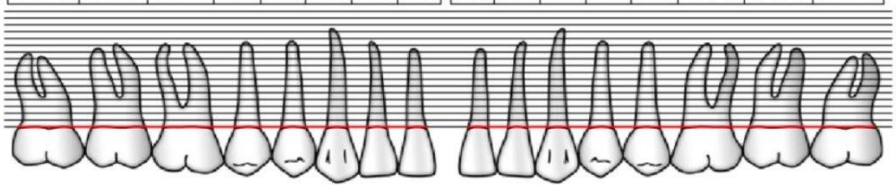


Gingival Margin	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Probing Depth	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Plaque																
Bleeding on Probing																
Furcation																
Note																

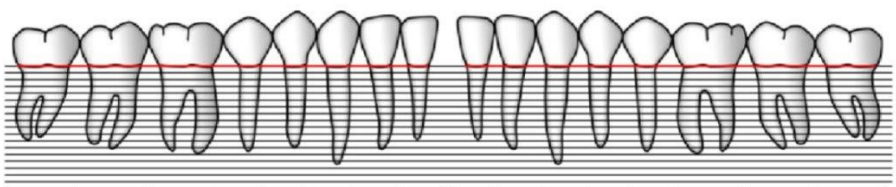
Mean Probing Depth = **0** mm Mean Attachment Level = **0** mm **0 %** Plaque **0 %** Bleeding on Probing

Note																
Furcation																
Bleeding on Probing																
Plaque																
Gingival Margin	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Probing Depth	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Lingual



Buccal



Gingival Margin	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Probing Depth	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Plaque																
Bleeding on Probing																
Furcation																
Implant																
Mobility	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

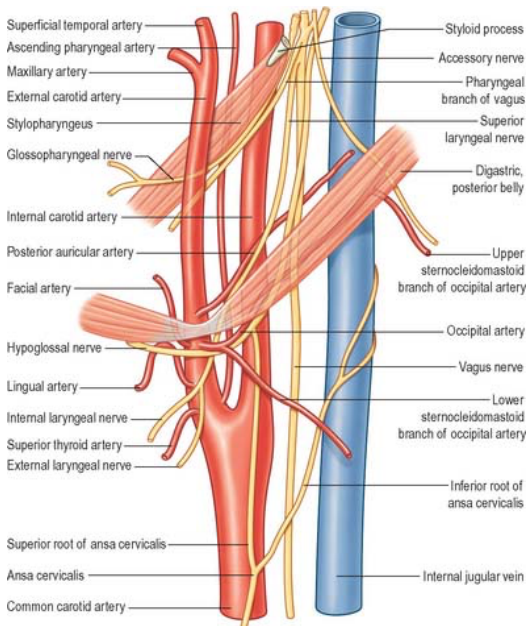
2.1.2 SUBGINGIVAL PLAQUE COLLECTION

A complete periodontal examination (pocket depth, clinical attachment level, plaque index, bleeding on probing), including radiographic orthopanoramic, was done by a single specialist (FC). The subgingival plaque samples were taken one or two days before patients underwent carotid endarterectomy. The deepest periodontal sites with periodontal depth ≧5mm were selected for microbial sampling. The teeth were gently dried with a sterile cotton swab. after removal of the supra gingival plaque, the sub gingival plaque samples were obtained with the help of a curette and were pooled for analysis.

2.1.3 SURGICAL INTERVENTION

Under local or general anesthesia a cervical incision is made parallel and anterior to the sternocleidomastoid and centered over the carotid bifurcation.

This incision can be extended proximally to the sternal notch for more proximal lesions of the common carotid artery (CCA) and distally to the mastoid process for higher exposure. Its upper end was angled posterior to the earlobe to avoid the parotid gland and the greater auricular nerve. The incision is carried down through the platysma, and the sternocleidomastoid is retracted laterally with self-retaining retractors. The internal jugular vein is visualized, and the carotid sheath is opened along the anterior border of the vein. The internal jugular vein is retracted laterally. Dissection is continued anterior to the CCA to keep from injuring the vagus nerve. The vagus nerve usually lies in a posterior lateral position within the carotid sheath but occasionally may spiral anteriorly, particularly in the lower end of the incision.



Attention should be paid to cranial nerves IX (glossopharyngeal nerve), X (vagus nerve), XI (accessory nerve), and XII (hypoglossal nerve), as well as the marginal mandibular branch of VII (facial nerve) and the rare nonrecurrent laryngeal nerve that comes directly off the vagus to innervate the vocal

cords. This nerve can cross anterior to the carotid artery and can be mistaken for a part of the ansa cervicalis; if it is inadvertently divided, cord paralysis results. A nonrecurrent laryngeal nerve is most often noted on the right side of the neck.

The CCA is mobilized proximal to the carotid lesion. Dissection is continued upward to isolate the external carotid artery (ECA). The internal carotid artery (ICA) is mobilized up to a point where the vessel is completely normal. Because the hypoglossal nerve may be injured by retraction, every effort was made to minimize traction on this nerve. Careful attention should also be given to the superior laryngeal nerve, which is usually located medial to the ICA. This nerve divides into external and internal branches that pass posterior to the superior thyroid artery and may be harmed while the surgeon is attempting to control either this vessel or the ICA. The glossopharyngeal nerve crosses the ICA near the base of the skull and is best protected by maintaining dissection close to the anterior surface of the ICA.

Control of the CCA is obtained proximal to the level of disease by surrounding the vessel with an umbilical tape. Once proximal control is obtained, dissection is continued distally around the ECA and its first branch, the superior thyroid artery. Subsequently, control is obtained distally at the ICA.

Throughout the dissection, it is important to minimize manipulation of the carotid artery so as to reduce the risk of embolization. Dissection must be carried out with extreme care taken to avoid injuring surrounding nerves, most notably the vagus and hypoglossal nerves. The ansa cervicalis, a branch of the hypoglossal nerve, may have to be divided to facilitate the dissection; this is acceptable.

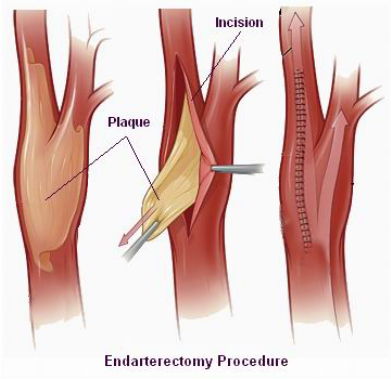
Heparin (5000-7000 U) is administered intravenously. The ICA, the CCA, and the ECA are occluded, in that order. An arteriotomy is made, starting anteriorly on the CCA proximal to the lesion and extending cephalad through the plaque opposite the flow divider, then continued into the ICA with Potts scissors. Distal to the plaque, the arteriotomy is extended until it reaches a point where the ICA is relatively normal.

When neurologic changes are noted during monitoring, a shunt is placed by inserting the distal end of the shunt into the normal ICA distal to the lesion. Back-bleeding the shunt clears any air or debris, and the proximal end of the shunt is then placed well into the CCA, proximal to the plaque. The endarterectomy proper is begun with a Penfield elevator. The optimal endarterectomy plane is that between the inner and outer medial layers. The cleavage plane is

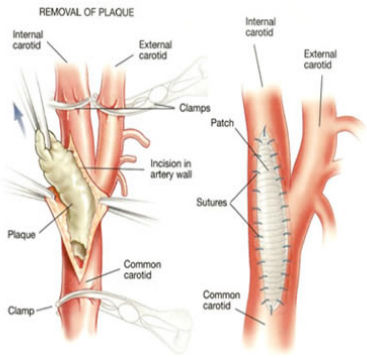
the key to the performance of an endarterectomy. The proximal endpoint is obtained by sharply dividing the plaque in the CCA. The plaque can be elevated under full vision while the endarterectomy is continued into the carotid bulb. Carotid plaque that extends a short distance into the ICA may be teased medially toward the origin of the ECA to achieve an adequate endpoint. The plaque can also be divided in the bulb so that the ICA and ECA endarterectomies can be conducted independently.

Once the plaque is divided, the device (clamp or loop) used to control the ECA is loosened, and an eversion endarterectomy is performed. In the ICA, the divided plaque is feathered so that a smooth taper is achieved in the transition to the normal distal intima. If a smooth distal taper is not achieved, placement of interrupted 7-0 monofilament tacking sutures may be necessary to secure the endpoint.

After completion of the endarterectomy, all residual debris and medial fibers are excised because of their potential contribution to embolization or hyperplastic restenosis. The endarterectomy surface is irrigated with heparinized saline solution to facilitate visualization and removal of all debris. Before the clamps are removed, flushing must be done from each direction. The ICA is unclamped last. As a rule, a conventional carotid endarterectomy is closed with a dacron patch or direct suture. Before closure is completed, heparinized saline solution is used to flush the ECA, the ICA, and the CCA. The shunt, if present, is removed, and the final few stitches are placed. Flow is then reestablished to the ECA and subsequently to the ICA.



DIRECT SUTURE



PATCH

Complete hemostasis is obtained. A closed suction drain is placed. The wounds are closed with routine technique.

2.1.3.1 CONSCIOUS PATIENT

The technique used for carotid endarterectomy in the conscious patient: with minimal premedication only, insufficient to cause drowsiness and inability to respond promptly to verbal commands, the patient is positioned on the operating table supine, the head turned away from

the operative side. A compressible squeaker toy is strapped in the palm of the hand opposite the side of operation. Cervical block anesthesia is administered injecting 0.5% marcaine.

2.1.4 COLLECTION OF ATHEROSCLEROTIC PLAQUE

After described endarterectomy the plaque was collected and placed in a vial with sterile saline solution, free of previous contaminating DNA. After surgery, all obtained plaque samples did not get in contact with any potential contaminants.

2.1.5 MICROBIOME DNA EXTRACTION

To purify bacterial DNA we implemented the PureLink Microbiome DNA purification kit (Invitrogen), following the manufacturer's instruction. The protocol was used both for DNA extraction from dental plaques as well as carotid plaques. Briefly, we added 800 uL of lysis buffer and transferred the whole content to the bead tube. Then, we added 100 uL of lysis enhancer, vortexed the suspension, and incubated for 10 minutes at 65°C. Subsequently, we homogenize by bead beating for 10 minutes on a vortex at maximum speed. At the end, we centrifuge the samples at 14'000 x g for 2 minutes, recovering 500 uL of supernatant. At this point, we added 900 uL of binding buffer and loaded onto a spin column-tube capable to capture the DNA. We washed the column with 500 uL of wash buffer, to remove all possible contaminants. We then proceeded with the elution step, adding 100 uL of elution buffer. We repeated this step twice to increase the DNA recovery. Finally, we quantify the total amount of DNA by the classical absorbance method. We stored the samples at -80°C until the analyses.

As expected the amount of DNA recovered from all the plaques was between 15 to 85 ng/μL. As shown in fig A the total amount of DNA in dental plaques was lower than carotid plaques. All the DNA extracted from carotid plaques was of bacterial origin, while DNA extracted from carotid plaques was of human and bacterial origin. Real Time PCR analysis evaluating 16s in carotid plaques, revealed that 5 samples out of 6 had detectable amount of bacterial DNA (fig B). Plot C shows the relative percentage of bacterial DNA in human carotid plaques, ranging from 9 to 25%.

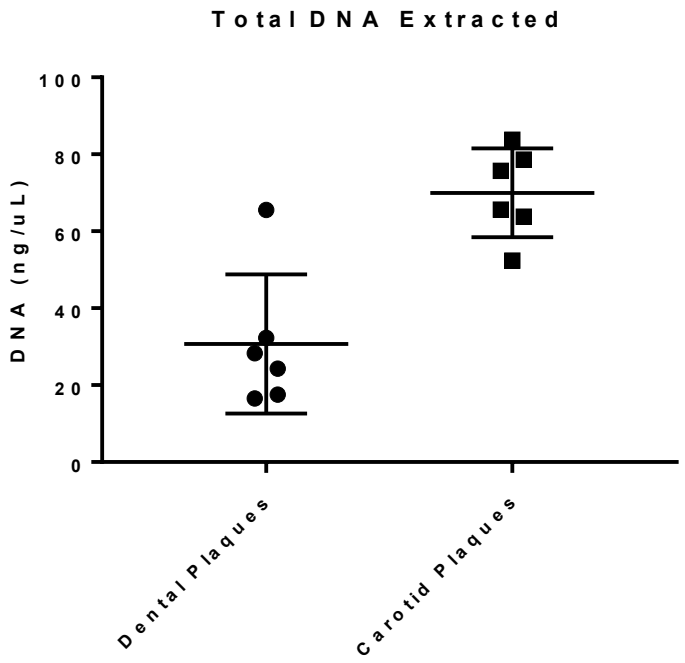


figure A

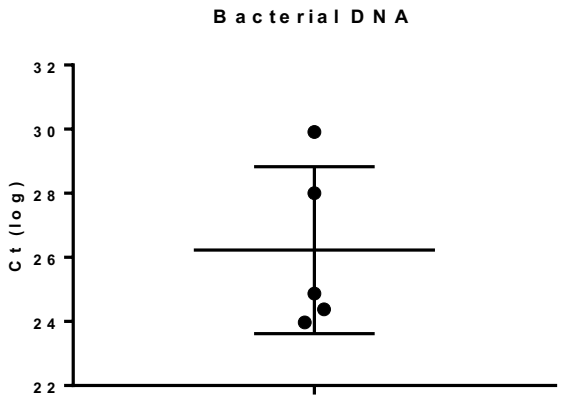


figure B

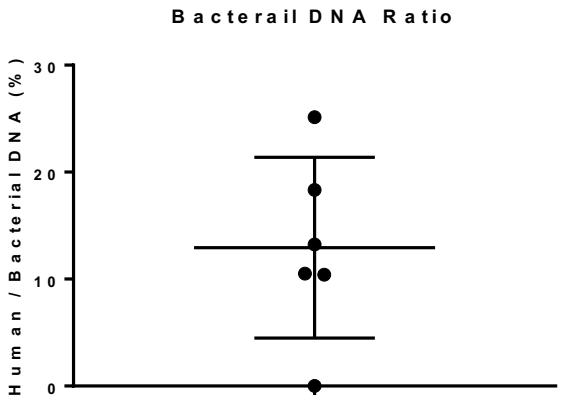


figure C

2.1.6 NEXT GENERATION SEQUENCING

The extracted DNA (from host and bacterial, not purified) was send to Eurofins Genomics, Ebersberg, Germany for 16S Microbiome profiling to identify the microbial community in our samples sequencing V1-V3 region.

The amount of bacterial DNA was very low in carotid plaques, Eurofins lab tried unsuccessfully to generate amplicons. Sequencing samples without an amplicon will result in no or very few reads that will give no reliable results. In addition samples without an amplicon will diluite the pool which will result in a weak coverage across all samples so we decided to sequencing only dental plaques.

Debarcoding Results				
Sample	#Read Pairs	Yield (Kbp)	%Q30	Mean Q
F01 PD	146,007	82,055	80,47	32,43
F02 PD	158,399	89,020	80,45	32,45
F03 PD	166,669	93,667	80,57	32,50
F04 PD	165,524	93,024	79,82	32,26
F05 PD	110,133	61,894	79,68	32,21
F06 PD	135,417	76,104	80,43	32,42
F01 PC	//	//	//	//
F02 PC	//	//	//	//
F03 PC	//	//	//	//
F04 PC	//	//	//	//
F05 PC	//	//	//	//
F06 PC	//	//	//	//

The sorting of sequencing reads according to barcodes and primer sequences has been performed with in-house scripts. Only read pairs where the expected 5' barcode and forward primer as well as the expected 3' barcode and reverse primer were found have been kept for further analysis. For the identification of barcode and primer sequences no mismatches were allowed. The "Debarcoding results" table provides various statistics describing the sorted reads.

2.1.7 MICROBIOME PROFILING

Prior to the microbiome analysis, raw reads were demultiplexed/debarcoded based on the unique forward and reverse sequencing indices and/or inline-barcode sequences. To preserve only high-quality reads, all reads with sequencing errors the barcode or primer sequences or with ambiguous bases ("N") were removed. Indices/barcodes as well as primer sequences were clipped from the reads. The remaining set of high-quality reads was processed using minimum entropy decomposition[78]. Minimum Entropy Decomposition (MED) provides a computationally efficient means to partition marker gene datasets into OTUs (Operational Taxonomic Units). Each OTU represents a distinct cluster with significant sequence divergence to any other cluster. By employing Shannon entropy, MED uses only the information-rich nucleotide positions across reads and iteratively partitions large datasets while omitting stochastic variation. The MED procedure outperformes classical, identity based clustering algorithms. Sequences can be partitioned based on relevant single nucleotide differences without being susceptible to random

sequencing errors. This allows a decomposition of sequence data sets with a single nucleotide resultion. Furthermore, the MED procedure identifies and filters random "noise" in the dataset. This includes singletons and putative chimeric sequences.

To assign taxonomic information to each OTU, BLAST alignments of cluster representative sequences to the NCBI sequence database were performed. A most specific taxonomic assignment for each OTU was then transferred from the set of best-matching reference sequences. Hereby, a sequence identity of 80% across at least 80% of the representative sequence was a minimal requirement for considering reference sequences.

Further processing of OTUs and taxonomic assignments was performed using the QIIME software package (version 1.8.0, http://qiime.org/). Abundances of bacteria and archaea taxonomic units were normalized using lineage-specific copy numbers of the relevant marker genes to improve estimates[79]. The amount of bacterial DNA is very low for half of samples send to Eurofins (carotid plaque samples) and no amplicon could be generated and sequencing samples without an amplicon will result in no or very few reads that will give no reliable results.

2.1.9 STATISTICS

This section summarizes the results of read preprocessing, OTU picking, and taxonomic assignment.

Summarized Statistics

Total number of input sequences	882,149	100%
Remaining sequences after preprocessing and quality filtering	882,149	100%
Total number of sequences assigned to OTUs	735,440	83,4%
Total number of sequences assigned to taxa	709,515	80,4%
Copy-number corrected total count	273,612	N/A
Total number of OTUs	3,027	100%
Number of OTUs assigned to taxa	2,915	96,3%
Sequences per sample assigned to OTUs	Min	90,525
	Max	142,101
	Median	126,542
	Mean	122,573
	Std. Dev.	17,342

The number of OTUs correlates with the diversity of the data set. Sequences that were considered as noise by the OTU picking algorithm were not assigned to an OTU. This includes singletons and putative chimeric sequences. The fraction of OTUs that could be assigned to taxa indicates how well the microbiome is represented in the used reference database. A copy-number correction was performed for bacterial species only, see Angly FE et al[79]. To do so, the number of reads assigned to a species was divided by the known or assumed copy-number of marker genes/regions. The resulting corrected total count may be significantly lower than the (raw) total number of assigned reads. After preprocessing, sequences were clipped to 270 bp length to remove low quality bases from the 3' end and to ensure that all sequences have the same length. The latter is crucial for the MED analysis.

Per Sample Statistics

	1)	2)	3)	4)	5)	6)
F01 PD	146,007	146,007	120,831	115,807	42,155	280
F02 PD	158,399	158,399	132,253	126,239	47,659	280
F03 PD	166,669	166,669	142,101	140,060	54,263	280
F04 PD	165,524	165,524	136,882	131,323	51,583	280
F05 PD	110,113	110,113	90,526	88,084	33,162	280
F06 PD	135,417	135,417	112,848	108,002	44,790	280
TOTAL	882,129	882,129	735,441	709,515	273,612	280

1) Input sequences. 2) Sequences after preprocessing. 3) Sequences assigned to OTUs. 4) Sequences assigned to taxa. 5) Count after lineage-specific copy-number correction. 6) Median sequence length after preprocessing and before clipping. This table can be found as file in the results directory. Please see the according section for details about result files.

2.2 RESULTS

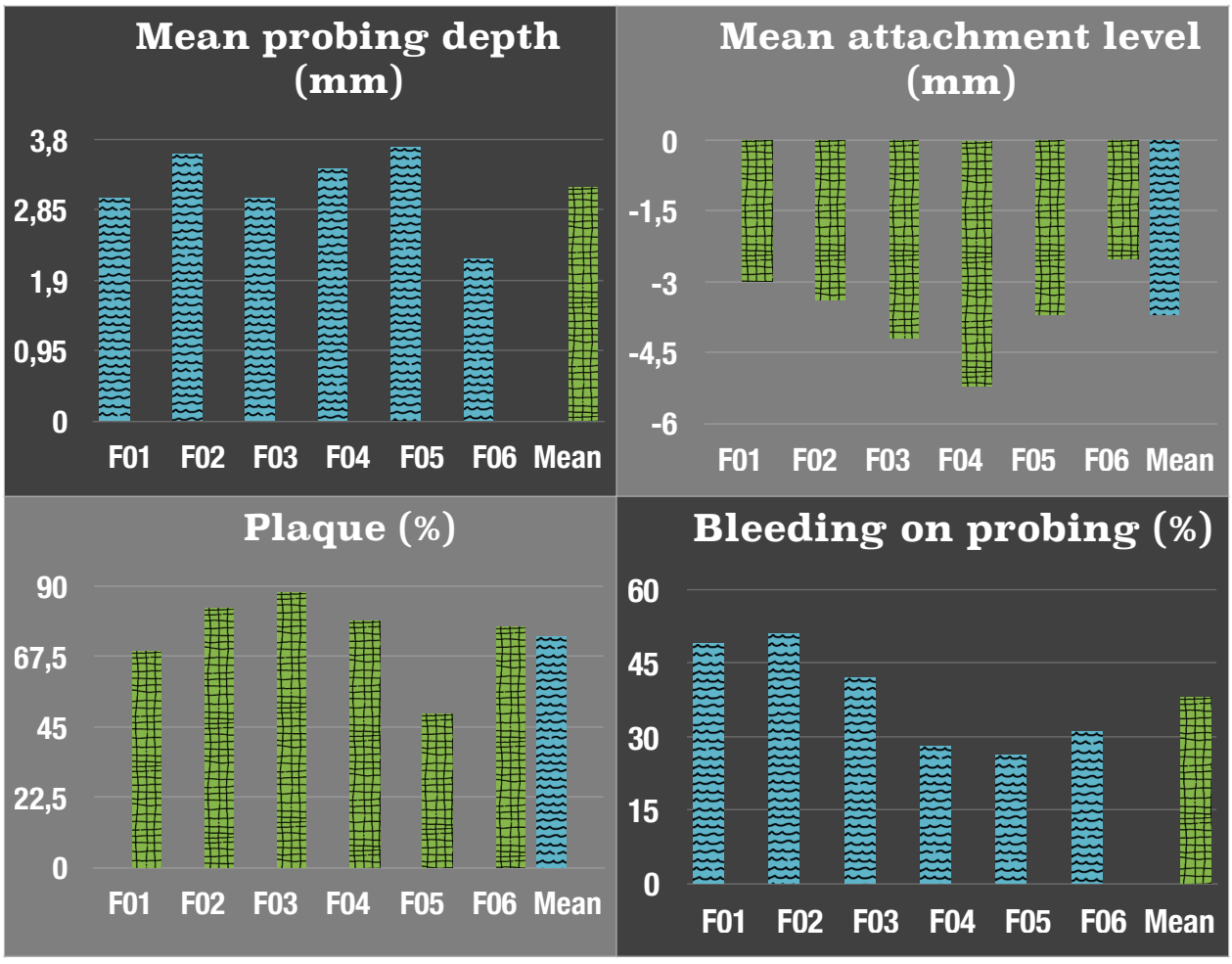
We enrolled six patients with a a mean age of 72±5.8 years, with a prevalence of male gender (67%), an half of obese patient with a mean BMI of 29.4. We don't enrolled smokers but we had 67% of previous smoker. About 67% of patients had concomitant coronary artery disease. Population data in following table.

Population characteristics

Age	72,2±5,8	**CAD**	67%
Male	67%	**previous CABG**	17%
BMI	29,4±2,4	**previous PCI**	33%

arterial hypertension	83%	**previous AMI**	17%
dislipidemia	100%	**EF**	61±2,8
Diabete	33%	**prepor statine**	83%
previous smoking	67%	**BNP**	103,4±106,2
Obesity	50%	**cholesterol mg/dL**	158±27,3
COPD	17%	**triglycerides mg/dL**	100,8±33,8
Creatinine mg/dL	1,2±0,67		

From periodontal chart we extrapolated some data: we have a mean probing depth of 3.1mm, a mean attachment level of -3.7mm, a mean plaque of 74% and a mean of bleeding on probing about 38%.



Periodontal chart results showed a moderate periodontitis with gingival inflammation.

About 50% of the surgical procedure was done under local anestesia, only in one patient was necessary to use a patch to close the carotid artery and in another patient we performed concomitant surgical myocardial revascularization. We had no death and no major morbidity.

Intraoperative characteristics

General Anesthesia	50%
Local anesthesia	50%
Right TEA	33%
Left TEA	67%
Patch	17%
concomitant CABG	17%

The DNA of at least one of the probed bacteria was detected in each subgingival samples. In carotid plaques not enough bacterial DNA was found to proceed with sequencing.

2.2.1 TAXONOMIC COMPOSITION OF ORAL PLAQUE SAMPLES

The following table provides an overview of the identified taxonomic units in each sample. Next to each sample name, the total number of reads of this sample that were assigned to OTUs is given. The most specific taxonomic units are listed with their taxonomy level and fraction (k...kingdom, p...phylum, c...class, o...order, f...family, g...genus, s...species). The most specific taxonomic unit is the lowest common taxonomic unit of the listed species. These species came up as best hits of the OTUs representative sequences during the database comparison.

All taxonomic units with less than 0.1% of reads are collapsed in the category "Other". If the representative sequence of an OTU had no significant database match, no taxonomic unit could be assigned. The total number of reads of these unassigned OTUs is stated as category "Unassigned". Depending on the type of analysis, some taxonomic units might be removed as they to not match the expected clade, e.g. eukaryotes in a bacterial microbiome analysis. The number of removed reads is stated as category "Filtered". If this category is not listed, no filtering was performed. Copy-number correction could be performed for bacterial species only. To do so, the assigned read count was divided by the known or assumed copy-number of marker genes/regions. If the listed normalized fraction and raw fraction are identical, either no copy-number correction factor was available in the database or the factor is exactly one. Copy-number information is only available for bacterial species, see Angly FE et al. (2014).

The following tables show the oral microbiome analysis of each patient containing a summarized list of identified taxonomic unit for each sample. The columns list all taxonomic unit with at least 0,1% of reads assigned to them. All taxonomic unit with less than 0,1% of reads are collapsed in

the category “other”. If the representative sequence of an OTU had no significant database match, no taxonomic unit could be assigned. The total number of reads of these unassigned OTUs is stated as category “unassigned”. Depending on the type of analysis some taxonomic units might be removed as they to not match the expected clade, e.g. eukaryotes in bacterial microbiome analysis. The number of removed reads is stated as category “filtered”.

LEGEND: K=Kingdom, P=Phylum, C=Class, O=Order, F=Family, G=Genus, S=Species

Patient F01				Patient F02			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Porphyromonas endodontalis	10,4%	3,8%	s	Campylobacter showae	4,9%	2,4%
s	Fusobacterium nucleatum	7,7%	13,4%	s	Lautropia mirabilis	4,1%	1,5%
g	Treponema	6,3%	3,9%	s	Actinomyces naeslundii	3,9%	2,0%
s	Alloprevotella tannerae	5,9%	6,2%	s	Porphyromonas endodontalis	3,2%	1,2%
s	Campylobacter rectus	5,2%	1,9%	g	Dysgonomonas	3,1%	1,2%
g	Fusobacterium	3,4%	5,9%	s	Prevotella intermedia	3,0%	2,8%
s	Prevotella sp. oral taxon 303	3,0%	3,0%	s	Aggregatibacter segnis	3,0%	3,5%
s	Fusobacterium sp. oral taxon 203	2,8%	4,9%	g	Prevotella	2,9%	3,0%
s	Prevotella oris	2,8%	2,7%	g	Streptococcus	2,8%	5,1%
s	Bosea sp. R-38307	2,7%	1,5%	s	Capnocytophaga sp. oral taxon 329	2,7%	1,9%
s	Parvimonas micra	2,3%	5,5%	s	Peptostreptococcus stomatis	2,7%	1,0%
s	Tannerella sp. oral taxon BU063	2,1%	1,5%	s	Fusobacterium hwasookii	2,2%	4,0%
s	Prevotella intermedia	2,0%	1,8%	s	Veillonella parvula	2,1%	5,3%
g	Prevotella	1,9%	1,9%	g	Actinomyces	2,1%	1,0%
s	Treponema socranskii	1,9%	1,2%	s	Prevotella sp. oral taxon 317	2,0%	2,0%
s	Corynebacterium matruchotii	1,6%	1,2%	s	Corynebacterium sp. oral taxon B00	1,6%	1,3%
s	Lachnoanaerobaculum saburreum	1,6%	1,5%	s	Capnocytophaga granulosa	1,6%	1,1%
s	Eubacterium brachy	1,5%	2,7%	g	Fusobacterium	1,5%	2,6%
s	Johnsonella ignava	1,3%	0,5%	s	Corynebacterium matruchotii	1,5%	1,2%
s	Rickettsia raoultii	1,1%	0,4%	g	Corynebacterium	1,4%	1,1%
s	Prevotella sp. oral taxon 317	1,1%	1,0%	s	Leptotrichia buccalis	1,4%	2,4%
s	Campylobacter gracilis	1,0%	0,4%	s	Propionibacterium propionicum	1,4%	1,1%
s	Capnocytophaga sp. oral taxon 329	0,9%	0,6%	s	Abiotrophia defectiva	1,3%	1,5%
s	Fretibacterium fastidiosum	0,9%	1,1%	s	Leptotrichia sp. oral taxon 225	1,3%	2,3%

Patient F01				Patient F02			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Fusobacterium sp. oral taxon A11	0,8%	1,4%	s	Neisseria sicca	1,2%	1,7%
s	Fusobacterium sp. oral taxon C10	0,8%	1,3%	s	Leptotrichia sp. oral taxon 392	1,2%	2,1%
s	Actinomyces meyeri	0,8%	0,4%	s	Treponema socranskii	1,2%	0,7%
s	Porphyromonas sp. oral taxon 279	0,7%	0,6%	g	Porphyromonas	1,1%	0,9%
s	Fretibacterium sp. feline oral taxon 223	0,7%	0,9%	s	Capnocytophaga sp. oral taxon B29	1,1%	0,7%
g	Streptococcus	0,7%	1,2%	s	Catonella morbi	1,0%	0,7%
s	Catonella morbi	0,7%	0,5%	s	Streptococcus sp. oral taxon C54	1,0%	1,8%
s	Leptotrichia sp. oral taxon 462	0,6%	1,1%	s	Neisseria elongata	0,9%	1,3%
s	Eikenella corrodens	0,6%	0,2%	s	Actinomyces sp. oral taxon 178	0,9%	0,4%
g	Corynebacterium	0,6%	0,4%	s	Actinomyces dentalis	0,8%	0,4%
s	Leptotrichia sp. oral taxon B57	0,6%	1,0%	s	Fusobacterium nucleatum	0,8%	1,4%
s	Atopobium rimae	0,6%	0,3%	o	Burkholderiales	0,8%	1,0%
s	Campylobacter showae	0,6%	0,3%	s	Porphyromonas gingivalis	0,7%	0,6%
s	Eubacterium sp. oral strain A35MT	0,5%	0,9%	s	Bosea sp. R-38307	0,7%	0,4%
f	Lachnospiraceae	0,5%	0,5%	s	Eikenella corrodens	0,7%	0,3%
s	Actinomyces sp. oral taxon 525	0,5%	0,2%	s	Capnocytophaga sputigena	0,7%	0,5%
g	Olsenella	0,4%	0,2%	s	Campylobacter gracilis	0,7%	0,3%
s	Tannerella sp. oral taxon 808	0,4%	0,3%	s	Prevotella melaninogenica	0,7%	0,5%
s	Peptostreptococcus stomatis	0,4%	0,2%	s	Prevotella oris	0,7%	0,7%
s	Leptotrichia hofstadii	0,4%	0,7%	s	Lachnoanaerobaculum cf. saburreum oral strain C27KA	0,7%	0,7%
s	Treponema amylovorum	0,4%	0,2%	s	Streptococcus sanguinis	0,7%	1,2%
s	Capnocytophaga granulosa	0,4%	0,3%	s	Atopobium rimae	0,7%	0,3%
g	Porphyromonas	0,4%	0,3%	s	Treponema sp. oral taxon 230	0,6%	0,4%
s	Mogibacterium timidum	0,4%	0,6%	s	Actinomyces sp. oral taxon 449	0,6%	0,3%
s	Capnocytophaga genosp. AHN8471	0,4%	0,2%	g	Capnocytophaga	0,6%	0,4%
s	Propionibacterium propionicum	0,3%	0,3%	g	Selenomonas	0,6%	1,4%
s	Treponema sp. oral taxon 270	0,3%	0,2%	g	Leptotrichia	0,6%	1,0%
g	Dysgonomonas	0,3%	0,1%	s	Capnocytophaga gingivalis	0,6%	0,4%
s	Treponema maltophilum	0,3%	0,2%	s	Peptostreptococcus sp. oral taxon 113	0,6%	0,2%
g	Aggregatibacter	0,3%	0,6%	s	Porphyromonas sp. oral taxon 278	0,6%	0,5%
s	Lachnoclostridium jejuense	0,3%	0,3%	s	Leptotrichia sp. oral taxon 498	0,6%	1,0%

Patient F01				Patient F02			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Oribacterium sp. oral taxon 372	0,3%	0,3%	f	Lachnospiraceae	0,6%	0,6%
s	Leptotrichia buccalis	0,3%	0,5%	s	Bergeyella sp. oral taxon 322	0,5%	0,6%
s	Capnocytophaga gingivalis	0,3%	0,2%	s	Actinomyces israelii	0,5%	0,3%
s	Selenomonas sputigena	0,3%	0,7%	s	Alloprevotella sp. oral taxon 473	0,5%	0,6%
c	Alphaproteobacteria	0,3%	0,2%	g	Olsenella	0,5%	0,2%
s	Prevotella sp. oral taxon 781	0,3%	0,3%	s	Rickettsia raoultii	0,4%	0,2%
s	Aggregatibacter sp. oral taxon 458	0,3%	0,5%	s	Porphyromonas catoniae	0,4%	0,3%
g	Leptotrichia	0,3%	0,4%	s	Prevotella sp. oral taxon 781	0,4%	0,4%
g	Selenomonas	0,3%	0,6%	s	Ruminococcus sp. 653	0,4%	0,3%
s	Actinomyces israelii	0,3%	0,1%	s	Streptococcus sp. oral taxon 071	0,4%	0,7%
s	Dialister invisus	0,3%	0,6%	s	Granulicatella adiacens	0,4%	0,1%
s	Actinobaculum sp. oral taxon 183	0,3%	0,1%	s	Leptotrichia sp. oral taxon 212	0,4%	0,7%
s	Selenomonas sp. oral taxon 146	0,2%	0,6%	g	Campylobacter	0,4%	0,2%
s	Streptococcus sp. oral taxon C54	0,2%	0,4%	s	Actinomyces cardiffensis	0,4%	0,2%
s	Prevotella sp. oral taxon 289	0,2%	0,2%	s	Tannerella sp. oral taxon BU063	0,3%	0,3%
s	Lautropia mirabilis	0,2%	0,1%	s	Treponema sp. IV:17B:D4B	0,3%	0,2%
s	Streptococcus cristatus	0,2%	0,4%	s	Fusobacterium canifelinum	0,3%	0,6%
s	Leptotrichia sp. oral taxon 463	0,2%	0,4%	s	Selenomonas noxia	0,3%	0,7%
g	Capnocytophaga	0,2%	0,1%	s	Neisseria sp. oral taxon 014	0,3%	0,4%
s	Actinomyces dentalis	0,2%	0,1%	s	Filifactor alocis	0,3%	0,4%
s	Actinomyces naeslundii	0,2%	0,1%	s	Peptoclostridium yurii	0,3%	0,8%
s	Prevotella micans	0,2%	0,2%	g	Bergeyella	0,3%	0,3%
s	Prevotella sp. oral taxon 300	0,2%	0,2%	s	Fretibacterium sp. feline oral taxon 223	0,3%	0,4%
s	Granulicatella adiacens	0,2%	0,1%	s	Tannerella forsythia	0,3%	0,2%
g	Alistipes	0,2%	0,1%	s	Leptotrichia wadei	0,2%	0,4%
s	Streptococcus mitis	0,2%	0,3%	s	Parvimonas sp. oral taxon 393	0,2%	0,6%
s	Prevotella enoeca	0,2%	0,2%	s	Veillonella dispar	0,2%	0,6%
s	Actinomyces gerencseriae	0,2%	0,1%	s	Peptostreptococcus sp. oral taxon 790	0,2%	0,1%
g	Brachymonas	0,2%	0,1%	g	Aggregatibacter	0,2%	0,4%
s	Bulleidia extructa	0,2%	0,4%	s	Leptotrichia sp. oral taxon 462	0,2%	0,3%
s	Campylobacter concisus	0,2%	0,1%	s	Selenomonas sputigena	0,2%	0,4%

Patient F01				Patient F02			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Slackia exigua	0,1%	0,1%	s	Leptotrichia sp. oral taxon 217	0,2%	0,3%
s	Capnocytophaga sputigena	0,1%	0,1%	s	Prevotella sp. oral taxon 306	0,2%	0,2%
s	Actinomyces sp. oral taxon 448	0,1%	0,1%	s	Fusobacterium periodonticum	0,2%	0,3%
f	Rhodospirillaceae	0,1%	0,2%	s	Actinomyces oricola	0,2%	0,1%
s	Selenomonas sp. oral taxon D75	0,1%	0,3%	s	Lachnoanaerobaculum saburreum	0,2%	0,2%
g	Actinomyces	0,1%	0,1%	g	Tannerella	0,2%	0,1%
s	Prevotella marshii	0,1%	0,1%	s	Streptococcus sp. oral taxon 070	0,2%	0,3%
s	Neisseria flava	0,1%	0,2%	s	Alloprevotella tannerae	0,2%	0,2%
s	Ruminococcus sp. 653	0,1%	0,1%	s	Herbinix sp. SD1D	0,2%	0,2%
g	Dialister	0,1%	0,3%	s	Actinomyces sp. oral taxon E91	0,2%	0,1%
s	Actinomyces georgiae	0,1%	0,1%	s	Selenomonas sp. oral taxon 126	0,2%	0,4%
s	Actinomyces cardiffensis	0,1%	0,0%	s	Prevotella oulorum	0,2%	0,2%
s	Tannerella forsythia	0,1%	0,1%	s	Prevotella baroniae	0,2%	0,2%
s	Schwartzia sp. canine oral taxon 042	0,1%	0,2%	s	Fretibacterium fastidiosum	0,1%	0,2%
	Other	4,2%		s	Prevotella sp. oral taxon 515	0,1%	0,1%
	Unassigned	5024		s	Solobacterium moorei	0,1%	0,3%
	Filtered	0		s	Desulfomicrobium orale	0,1%	0,1%
				s	Prevotella veroralis	0,1%	0,1%
				g	Parvimonas	0,1%	0,3%
				s	Streptococcus cristatus	0,1%	0,2%
				g	Dialister	0,1%	0,3%
				s	Dialister invisus	0,1%	0,3%
				s	Prevotella sp. oral taxon 289	0,1%	0,1%
				s	Parvimonas micra	0,1%	0,2%
				s	Actinomyces massiliensis	0,1%	0,1%
				s	Eggerthia catenaformis	0,1%	0,3%
				s	Mycoplasma faucium	0,1%	0,0%
					Other	3,7%	
					Unassigned	6014	
					Filtered	0	

Patient F03				Patient F04			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Corynebacterium matruchotii	22,9%	18,8%	s	Corynebacterium matruchotii	5,8%	4,8%
s	Streptococcus cristatus	3,7%	7,0%	s	Rickettsia raoultii	4,8%	1,9%
s	Granulicatella adiacens	3,6%	1,4%	s	Porphyromonas endodontalis	3,6%	1,4%
g	Fusobacterium	3,6%	6,5%	s	Fusobacterium nucleatum	3,2%	5,8%
s	Capnocytophaga granulosa	3,4%	2,5%	s	Peptostreptococcus sp. oral taxon 113	3%	1,2%
s	Actinomyces naeslundii	3,3%	1,7%	s	Porphyromonas gingivalis	2,7%	2,3%
s	Leptotrichia wadei	3,0%	5,6%	g	Capnocytophaga	2,3%	1,7%
g	Corynebacterium	2,7%	2,2%	s	Capnocytophaga granulosa	2,3%	1,7%
s	Neisseria elongata	2,6%	3,7%	s	Actinomyces israelii	2%	1,1%
s	Corynebacterium sp. oral taxon B00	2,5%	2,1%	g	Fusobacterium	2%	3,7%
s	Campylobacter showae	2,5%	1,2%	g	Dysgonomonas	2%	0,8%
g	Actinomyces	2,4%	1,2%	g	Treponema	2%	1,3%
s	Lachnoanaerobaculum saburreum	1,9%	1,9%	s	Campylobacter gracilis	2%	1%
s	Capnocytophaga sp. oral taxon 332	1,7%	1,3%	s	Leptotrichia hofstadii	1,8%	3,4%
g	Leptotrichia	1,7%	3,1%	s	Actinomyces sp. oral taxon 178	1,8%	0,9%
s	Capnocytophaga sp. oral taxon 329	1,6%	1,2%	s	Actinobaculum sp. oral taxon 183	1,8%	1,1%
s	Tannerella sp. oral taxon BU063	1,5%	1,2%	s	Leptotrichia buccalis	1,8%	3,3%
s	Porphyromonas sp. oral taxon 279	1,4%	1,2%	s	Streptococcus mutans	1,7%	3,2%
g	Porphyromonas	1,2%	1,1%	g	Selenomonas	1,6%	4,1%
s	Actinomyces sp. oral taxon 170	1,2%	0,6%	s	Alloprevotella tannerae	1,4%	1,6%
s	Leptotrichia sp. oral taxon 225	1,1%	2,1%	s	Capnocytophaga sp. oral taxon 329	1,4%	1%
s	Johnsonella sp. oral taxon 166	1,1%	0,4%	s	Pseudoramibacter alactolyticus	1,3%	0,5%
g	Prevotella	1,0%	1,1%	g	Prevotella	1,3%	1,4%
s	Capnocytophaga gingivalis	1,0%	0,7%	s	Prevotella sp. oral taxon 317	1,2%	1,2%
s	Eikenella corrodens	0,9%	0,4%	s	Streptococcus sp. oral taxon C54	1,1%	2,1%
s	Actinomyces sp. 'ARUP UnID 58'	0,9%	0,5%	s	Actinomyces sp. oral taxon 449	1,1%	0,6%
g	Streptococcus	0,9%	1,6%	s	Leptotrichia wadei	1,1%	2%
s	Campylobacter gracilis	0,8%	0,4%	s	Porphyromonas catoniae	1,1%	0,9%
s	Lautropia mirabilis	0,8%	0,3%	s	Lautropia mirabilis	1%	0,4%

Patient F03				Patient F04			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Capnocytophaga sp. oral taxon 336	0,8%	0,6%	s	Fusobacterium sp. oral taxon A11	0,9%	1,7%
s	Actinomyces sp. oral taxon 178	0,8%	0,4%	s	Campylobacter showae	0,9%	0,4%
g	Capnocytophaga	0,8%	0,6%	s	Treponema denticola	0,9%	0,6%
s	Streptococcus sp. oral taxon C54	0,8%	1,4%	s	Veillonella parvula	0,8%	2,1%
s	Prevotella sp. 8404125	0,7%	0,8%	s	Actinomyces sp. oral taxon D05	0,8%	0,4%
g	Selenomonas	0,7%	1,7%	s	Treponema socranskii	0,8%	0,5%
s	Prevotella sp. oral taxon 317	0,6%	0,6%	s	Prevotella oris	0,8%	0,8%
s	Fusobacterium nucleatum	0,6%	1,0%	s	Capnocytophaga gingivalis	0,8%	0,6%
s	Veillonella parvula	0,5%	1,3%	s	Prevotella sp. oral taxon 303	0,8%	0,8%
s	Actinomyces dentalis	0,5%	0,3%	g	Streptococcus	0,8%	1,4%
s	Actinomyces sp. oral taxon E91	0,5%	0,2%	g	Olsenella	0,8%	0,4%
s	Campylobacter concisus	0,5%	0,2%	s	Lachnoanaerobaculum saburreum	0,7%	0,8%
s	Propionibacterium propionicum	0,5%	0,4%	s	Eikenella corrodens	0,7%	0,3%
s	Fusobacterium periodonticum	0,5%	0,8%	s	Actinomyces sp. 'ARUP UnID 71'	0,7%	0,4%
s	Streptococcus sanguinis	0,5%	0,9%	s	Aggregatibacter segnis	0,7%	0,9%
s	Selenomonas noxia	0,5%	1,2%	g	Tannerella	0,7%	0,6%
f	Lachnospiraceae	0,5%	0,5%	s	Campylobacter sp. oral taxon G43	0,7%	0,3%
s	Leptotrichia hofstadii	0,4%	0,8%	s	Streptococcus cristatus	0,6%	1,2%
s	Leptotrichia sp. oral taxon 215	0,4%	0,7%	s	Streptococcus mitis	0,6%	1,1%
s	Prevotella intermedia	0,3%	0,3%	s	Streptococcus sp. oral taxon 070	0,6%	1,1%
s	Leptotrichia sp. oral taxon 217	0,3%	0,6%	s	Selenomonas sp. oral taxon G00	0,5%	1,4%
s	Streptococcus sp. oral taxon 071	0,3%	0,6%	s	Tannerella forsythia	0,5%	0,4%
s	Actinomyces israelii	0,3%	0,2%	s	Fretibacterium sp. feline oral taxon 223	0,5%	0,7%
s	Alloprevotella sp. oral taxon 473	0,3%	0,4%	s	Capnocytophaga sputigena	0,5%	0,4%
s	Kingella oralis	0,3%	0,1%	s	Desulfomicrobium orale	0,5%	0,4%
s	Aggregatibacter sp. oral taxon 458	0,3%	0,6%	s	Kingella oralis	0,5%	0,2%
s	Porphyromonas endodontalis	0,3%	0,1%	s	Capnocytophaga sp. oral taxon 332	0,5%	0,4%
s	Porphyromonas sp. oral taxon 277	0,3%	0,2%	s	Actinomyces massiliensis	0,5%	0,3%
o	Lactobacillales	0,3%	0,4%	s	Porphyromonas sp. oral taxon 278	0,5%	0,4%
s	Lachnoclostridium jejuense	0,3%	0,3%	s	Leptotrichia sp. oral taxon 217	0,5%	0,9%
s	Actinobaculum sp. oral taxon 183	0,3%	0,2%	s	Odoribacter denticanis	0,5%	0,4%

Patient F03				Patient F04			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
s	Prevotella sp. oral taxon 781	0,3%	0,3%	s	Lachnoclostridium phytofermentans	0,5%	0,5%
g	Alistipes	0,3%	0,2%	s	Tannerella sp. oral taxon BU063	0,4%	0,3%
s	Leptotrichia buccalis	0,3%	0,5%	s	Corynebacterium sp. oral taxon B00	0,4%	0,4%
s	Kingella denitrificans	0,3%	0,1%	s	Neisseria bacilliformis	0,4%	0,6%
g	Olsenella	0,3%	0,1%	s	Parasporobacterium paucivorans	0,4%	0,4%
s	Actinomyces sp. 'ARUP UnID 56'	0,3%	0,1%	s	Campylobacter concisus	0,4%	0,2%
s	Actinomyces sp. oral taxon E63	0,2%	0,1%	s	Capnocytophaga sp. oral taxon B29	0,4%	0,3%
g	Dysgonomonas	0,2%	0,1%	g	Actinomyces	0,4%	0,2%
s	Bergeyella sp. oral taxon 322	0,2%	0,2%	s	Fusobacterium sp. oral taxon C10	0,4%	0,7%
s	Leptotrichia genomosp. C1	0,2%	0,4%	s	Actinomyces georgiae	0,4%	0,2%
s	Prevotella sp. oral taxon 289	0,2%	0,2%	s	Capnocytophaga genosp. AHN8471	0,3%	0,2%
g	Treponema	0,2%	0,1%	s	Actinomyces sp. oral taxon 414	0,3%	0,2%
s	Aggregatibacter segnis	0,2%	0,2%	s	Prevotella melaninogenica	0,3%	0,3%
s	Treponema sp. oral taxon G85	0,2%	0,1%	s	Capnocytophaga sp. AHN9576	0,3%	0,2%
s	Actinomyces sp. oral taxon 414	0,2%	0,1%	s	Lachnoclostridium jejuense	0,3%	0,3%
s	Catonella morbi	0,2%	0,1%	s	Prevotella sp. oral taxon 306	0,3%	0,3%
g	Campylobacter	0,2%	0,1%	s	Leptotrichia shahii	0,3%	0,6%
s	Actinomyces sp. oral taxon D50	0,2%	0,1%	s	Prevotella oulorum	0,3%	0,3%
s	Porphyromonas sp. oral taxon 284	0,2%	0,2%	s	Prevotella sp. 8404125	0,3%	0,3%
s	Capnocytophaga sp.	0,2%	0,1%	s	Streptococcus anginosus	0,3%	0,1%
s	Porphyromonas sp. oral taxon B43	0,2%	0,1%	s	Actinomyces dentalis	0,3%	0,1%
g	Veillonella	0,2%	0,4%	s	Capnocytophaga sp. oral taxon E54	0,3%	0,2%
s	Streptococcus mitis	0,2%	0,3%	s	Treponema sp. canine oral taxon 395	0,3%	0,2%
s	Prevotella sp. oral taxon 475	0,2%	0,2%	s	Treponema vincentii	0,3%	0,2%
s	Streptococcus sp. oral taxon 064	0,1%	0,3%	s	Parvimonas micra	0,3%	0,6%
g	Cardiobacterium	0,1%	0,2%	s	Catonella morbi	0,3%	0,2%
s	Corynebacterium durum	0,1%	0,1%	s	Johnsonella ignava	0,2%	0,1%
s	Treponema socranskii	0,1%	0,1%	s	Prevotella veroralis	0,2%	0,3%
g	Oribacterium	0,1%	0,1%	s	Streptococcus gordonii	0,2%	0,5%
g	Neisseria	0,1%	0,2%	s	Campylobacter curvus	0,2%	0,1%
g	Aggregatibacter	0,1%	0,2%	s	Moryella sp. KHD1	0,2%	0,2%

Patient F03				Patient F04			
Taxonomic Level	**Taxonomic Unit**	**Fraction**	**Uncorrected**	**Taxonomic Level**	**Taxonomic Unit**	**Fraction**	**Uncorrected**
s	Selenomonas sp. oral taxon D75	0,1%	0,3%	g	Porphyromonas	0,2%	0,2%
s	Cardiobacterium hominis	0,1%	0,1%	s	Treponema maltophilum	0,2%	0,1%
	Other	2,3%		s	Fusobacterium sp. oral taxon 203	0,2%	0,4%
	Unassigned	2041		s	Selenomonas sp. oral taxon 133	0,2%	0,6%
	Filtered	0		s	Selenomonas sp. oral taxon 140	0,2%	0,6%
				s	Propionibacterium propionicum	0,2%	0,2%
				s	Granulicatella adiacens	0,2%	0,1%
				s	Actinomyces sp. oral taxon 170	0,2%	0,1%
				s	Capnocytophaga sp. FVAMC 7623	0,2%	0,2%
				s	Actinomyces gerencseriae	0,2%	0,1%
				s	Mycoplasma salivarium	0,2%	0,1%
				g	Corynebacterium	0,2%	0,1%
				s	Eubacterium brachy	0,2%	0,4%
				s	Filifactor alocis	0,2%	0,3%
				s	Leptotrichia sp. oral taxon 392	0,2%	0,3%
				s	Fusobacterium periodonticum	0,2%	0,3%
				s	Leptotrichia trevisanii	0,2%	0,3%
				s	Sporobacterium olearium	0,2%	0,2%
				s	Streptococcus sp. oral taxon 058	0,2%	0,3%
				s	Prevotella maculosa	0,2%	0,2%
				s	Johnsonella sp. oral taxon 166	0,2%	0,1%
				s	Desulfobulbus sp. oral taxon 041	0,2%	0,1%
				s	Selenomonas infelix	0,1%	0,4%
				s	Actinomyces sp. oral taxon 448	0,1%	0,1%
				s	Selenomonas sputigena	0,1%	0,3%
				s	Bacteroides heparinolyticus	0,1%	0,2%
				s	Prevotella salivae	0,1%	0,1%
				s	Prevotella sp. oral taxon 300	0,1%	0,1%
				s	Campylobacter rectus	0,1%	0%
				s	Cardiobacterium hominis	0,1%	0,1%
				s	Lachnoanaerobaculum cf. saburreum oral strain C27KA	0,1%	0,1%

Patient F03				Patient F04			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
				s	Actinomyces meyeri	0,1%	0,1%
				s	Prevotella sp. oral taxon 289	0,1%	0,1%
				s	Actinomyces naeslundii	0,1%	0,1%
				s	Flexilinea flocculi	0,1%	0,1%
				s	Mogibacterium timidum	0,1%	0,2%
				s	Selenomonas sp. oral taxon E57	0,1%	0,3%
					Other	3,2%	
					Unassigned	5559	
					Filtered	0	

PATIENT F05				PATIENT F06			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
s	Streptococcus anginosus	13,2%	5,0%	s	Campylobacter showae	6,7%	3,5%
g	Prevotella	7,9%	8,0%	g	Dysgonomonas	4,5%	1,9%
g	Streptococcus	4,1%	7,6%	s	Porphyromonas gingivalis	4,2%	3,8%
g	Actinomyces	3,4%	1,7%	g	Streptococcus	3,2%	6,4%
s	Aggregatibacter segnis	3,0%	3,5%	s	Porphyromonas endodontalis	2,9%	1,2%
s	Veillonella parvula	2,8%	7,0%	s	Lautropia mirabilis	2,4%	1,0%
g	Campylobacter	2,8%	1,4%	s	Capnocytophaga sp. oral taxon 329	2,3%	1,8%
s	Peptostreptococcus stomatis	2,6%	1,0%	g	Corynebacterium	2,3%	2,0%
s	Alloprevotella tannerae	2,6%	2,9%	s	Corynebacterium sp. oral taxon B00	2,2%	1,9%
s	Leptotrichia sp. oral taxon 498	2,3%	4,1%	s	Johnsonella sp. oral taxon 166	2,1%	0,9%
g	Fusobacterium	2,2%	3,9%	g	Leptotrichia	1,9%	3,7%
s	Porphyromonas gingivalis	2,0%	1,6%	g	Fusobacterium	1,8%	3,5%
s	Campylobacter gracilis	2,0%	0,9%	s	Fusobacterium nucleatum	1,8%	3,4%
g	Dysgonomonas	1,8%	0,7%	s	Actinomyces sp. oral taxon G84	1,7%	0,9%
s	Corynebacterium matruchotii	1,7%	1,4%	g	Treponema	1,6%	1,1%
s	Rickettsia raoultii	1,6%	0,6%	g	Alistipes	1,5%	1,0%
s	Tannerella sp. oral taxon BU063	1,6%	1,2%	s	Neisseria elongata	1,5%	2,3%
s	Neisseria elongata	1,6%	2,2%	s	Prevotella intermedia	1,5%	1,5%
s	Prevotella sp. oral taxon 317	1,5%	1,5%	o	Burkholderiales	1,4%	2,0%

PATIENT F05				PATIENT F06			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
s	Capnocytophaga sp. oral taxon 329	1,5%	1,0%	s	Actinomyces naeslundii	1,4%	0,8%
s	Fusobacterium nucleatum	1,3%	2,2%	s	Propionibacterium propionicum	1,4%	1,2%
s	Campylobacter showae	1,3%	0,6%	s	Eikenella corrodens	1,3%	0,5%
g	Olsenella	1,2%	0,6%	s	Lautropia sp. oral taxon A94	1,2%	0,5%
s	Prevotella oris	1,0%	1,0%	s	Capnocytophaga gingivalis	1,2%	0,9%
g	Selenomonas	1,0%	2,5%	s	Leptotrichia buccalis	1,2%	2,3%
s	Porphyromonas endodontalis	1,0%	0,4%	s	Lachnoanaerobaculum saburreum	1,2%	1,3%
s	Actinomyces naeslundii	1,0%	0,5%	s	Catonella morbi	1,1%	0,9%
s	Leptotrichia wadei	0,9%	1,7%	s	Peptostreptococcus stomatis	1,1%	0,5%
s	Granulicatella adiacens	0,9%	0,4%	s	Leptotrichia sp. oral taxon 392	1,0%	2,0%
s	Atopobium rimae	0,9%	0,4%	g	Tannerella	1,0%	0,8%
g	Neisseria	0,8%	1,1%	s	Atopobium rimae	1,0%	0,5%
s	Eikenella corrodens	0,8%	0,3%	s	Streptococcus sanguinis	0,9%	1,8%
s	Treponema socranskii	0,7%	0,5%	s	Rickettsia raoultii	0,8%	0,3%
g	Treponema	0,7%	0,5%	s	Actinomyces sp. oral taxon 525	0,8%	0,4%
s	Capnocytophaga granulosa	0,7%	0,5%	s	Prevotella sp. oral taxon 781	0,8%	0,8%
s	Lachnoanaerobaculum cf. saburreum oral strain C27KA	0,7%	0,7%	s	Actinomyces israelii	0,7%	0,4%
s	Prevotella baroniae	0,7%	0,7%	g	Prevotella	0,7%	0,8%
s	Leptotrichia sp. oral taxon 392	0,6%	1,1%	s	Treponema socranskii	0,7%	0,5%
f	Lachnospiraceae	0,6%	0,6%	s	Prevotella sp. oral taxon 472	0,7%	0,8%
s	Leptotrichia sp. 'ARUP UnID 389'	0,6%	1,0%	s	Campylobacter gracilis	0,7%	0,4%
s	Streptococcus sp. oral taxon C54	0,5%	0,9%	g	Selenomonas	0,7%	1,9%
s	Capnocytophaga sp. AHN9576	0,5%	0,3%	g	Capnocytophaga	0,7%	0,5%
s	Parvimonas micra	0,5%	1,1%	s	Actinomyces dentalis	0,7%	0,4%
s	Catonella morbi	0,5%	0,3%	s	Actinomyces sp. oral taxon 178	0,7%	0,4%
s	Actinomyces sp. oral taxon 446	0,4%	0,2%	f	Lachnospiraceae	0,7%	0,7%
s	Anaeroglobus geminatus	0,4%	1,1%	s	Prevotella sp. oral taxon 317	0,7%	0,7%
g	Porphyromonas	0,4%	0,3%	s	Actinomyces oricola	0,6%	0,4%
f	Rhodospirillaceae	0,4%	0,6%	s	Lachnoclostridium jejuense	0,6%	0,7%
s	Porphyromonas sp. oral taxon 278	0,4%	0,3%	s	Lachnoanaerobaculum cf. saburreum oral strain C27KA	0,6%	0,7%
s	Actinomyces israelii	0,4%	0,2%	s	Capnocytophaga sp. 'ARUP UnID 182'	0,6%	0,4%

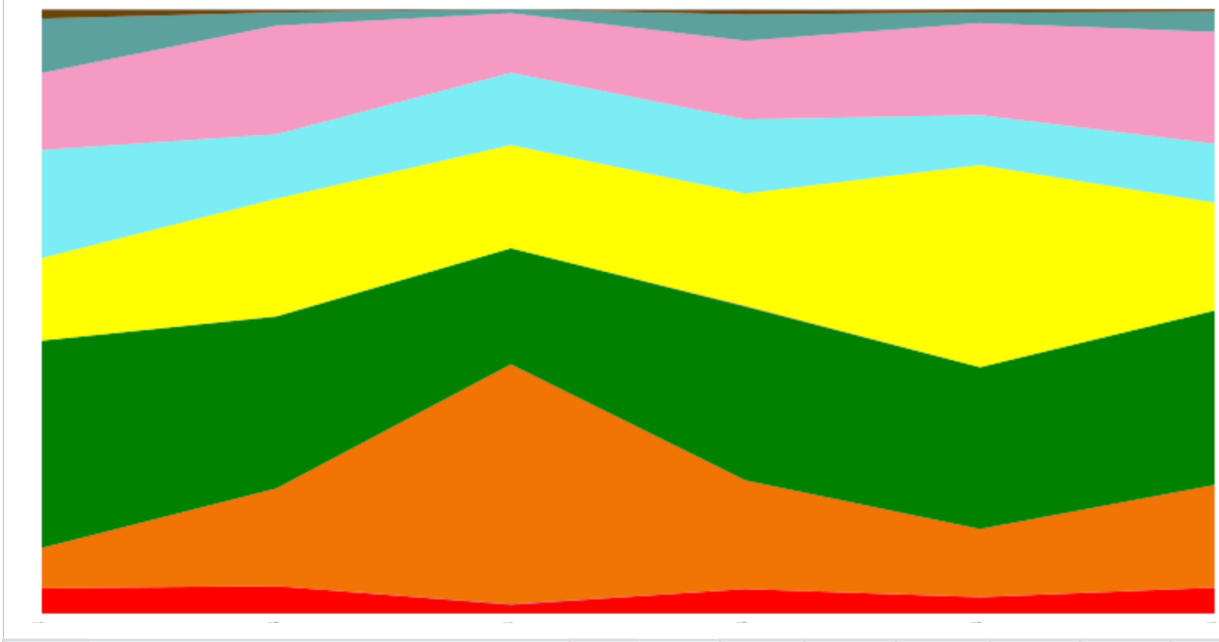
PATIENT F05				PATIENT F06			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
g	Corynebacterium	0,4%	0,3%	s	Selenomonas noxia	0,6%	1,6%
s	Selenomonas noxia	0,4%	0,9%	g	Campylobacter	0,6%	0,3%
s	Actinomyces sp. 'ARUP UnID 56'	0,3%	0,2%	s	Actinomyces sp. oral taxon E91	0,5%	0,3%
s	Capnocytophaga sp. oral taxon E54	0,3%	0,2%	g	Olsenella	0,5%	0,3%
s	Selenomonas sputigena	0,3%	0,8%	f	Rhodospirillaceae	0,5%	0,7%
s	Prevotella sp. oral taxon 306	0,3%	0,3%	s	Actinomyces sp. oral taxon 449	0,5%	0,3%
s	Magnetofaba australis	0,3%	0,3%	s	Capnocytophaga granulosa	0,5%	0,4%
s	Lachnoanaerobaculum saburreum	0,3%	0,3%	g	Actinomyces	0,5%	0,3%
s	Fretibacterium sp. feline oral taxon 223	0,3%	0,4%	s	Leptotrichia sp. oral taxon B57	0,5%	0,9%
s	Prevotella veroralis	0,3%	0,3%	s	Actinomyces sp. oral taxon 414	0,4%	0,2%
s	Bergeyella sp. oral taxon 322	0,3%	0,3%	s	Granulicatella adiacens	0,4%	0,2%
s	Streptococcus sp. oral taxon 058	0,3%	0,5%	s	Lachnoclostridium phytofermentans	0,4%	0,5%
s	Streptococcus cristatus	0,3%	0,5%	s	Porphyromonas catoniae	0,4%	0,4%
s	Actinomyces sp. 'ARUP UnID 92'	0,3%	0,1%	s	Fusobacterium periodonticum	0,4%	0,7%
s	Prevotella marshii	0,3%	0,3%	s	Aggregatibacter segnis	0,4%	0,5%
s	Aggregatibacter sp. oral taxon 513	0,3%	0,5%	s	Treponema maltophilum	0,4%	0,3%
s	Solobacterium moorei	0,3%	0,6%	s	Leptotrichia wadei	0,4%	0,7%
s	Selenomonas infelix	0,2%	0,6%	s	Magnetofaba australis	0,4%	0,3%
s	Slackia exigua	0,2%	0,1%	s	Prevotella baroniae	0,3%	0,4%
s	Prevotella micans	0,2%	0,2%	s	Leptotrichia sp. oral taxon 212	0,3%	0,7%
s	Selenomonas dianae	0,2%	0,6%	s	Streptococcus sp. oral taxon C54	0,3%	0,6%
s	Fretibacterium fastidiosum	0,2%	0,3%	s	Capnocytophaga sp. AHN10044	0,3%	0,2%
s	Alloprevotella rava	0,2%	0,2%	s	Fretibacterium sp. feline oral taxon 223	0,3%	0,5%
s	Mogibacterium timidum	0,2%	0,4%	s	Capnocytophaga sputigena	0,3%	0,2%
s	Parasporobacterium paucivorans	0,2%	0,2%	s	Campylobacter concisus	0,3%	0,2%
g	Megasphaera	0,2%	0,6%	s	Prevotella oulorum	0,3%	0,3%
s	Eubacterium sp. oral strain A35MT	0,2%	0,4%	s	Prevotella sp. oral taxon 526	0,3%	0,3%
g	Dialister	0,2%	0,5%	s	Fusobacterium hwasookii	0,3%	0,5%
s	Capnocytophaga gingivalis	0,2%	0,1%	g	Bergeyella	0,3%	0,3%
s	Actinomyces dentalis	0,2%	0,1%	s	Capnocytophaga sp. oral taxon 323	0,3%	0,2%
s	Kingella denitrificans	0,2%	0,1%	s	Actinomyces massiliensis	0,2%	0,1%

PATIENT F05				PATIENT F06			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
s	Prevotella oralis	0,2%	0,2%	s	Tannerella sp. oral taxon BU063	0,2%	0,2%
s	Prevotella sp. oral taxon 781	0,2%	0,2%	s	Actinomyces sp. oral taxon 172	0,2%	0,1%
s	Streptococcus gordonii	0,2%	0,3%	s	Bergeyella sp. AF14	0,2%	0,3%
s	Filifactor alocis	0,2%	0,3%	s	Prevotella sp. oral taxon C29	0,2%	0,3%
s	Dialister invisus	0,2%	0,4%	s	Peptostreptococcus sp. oral taxon 113	0,2%	0,1%
s	Actinomyces georgiae	0,2%	0,1%	s	Actinomyces cardiffensis	0,2%	0,1%
s	Herbinix sp. SD1D	0,2%	0,2%	s	Prevotella oris	0,2%	0,2%
s	Kingella oralis	0,2%	0,1%	s	Campylobacter sp. oral taxon 044	0,2%	0,1%
s	Actinomyces sp. oral taxon E91	0,2%	0,1%	s	Eubacterium sp. 'Smarlab BioMol-2301231'	0,2%	0,4%
g	Atopobium	0,2%	0,1%	s	Selenomonas infelix	0,2%	0,5%
s	Streptococcus mitis	0,1%	0,3%	s	Actinobaculum sp. oral taxon 183	0,2%	0,1%
g	Veillonella	0,1%	0,4%	s	Veillonella parvula	0,2%	0,4%
s	Actinomyces massiliensis	0,1%	0,1%	s	Bosea sp. R-38307	0,2%	0,1%
s	Prevotella maculosa	0,1%	0,1%	s	Prevotella loescheii	0,2%	0,2%
s	Leptotrichia sp. oral taxon 215	0,1%	0,2%	s	Mucinivorans hirudinis	0,2%	0,1%
s	Propionibacterium propionicum	0,1%	0,1%	s	Treponema vincentii	0,2%	0,1%
s	Selenomonas sp. oral taxon E83	0,1%	0,3%	s	Prevotella sp. oral taxon 289	0,2%	0,2%
s	Leptotrichia sp. oral taxon 212	0,1%	0,2%	s	Herbinix sp. SD1D	0,2%	0,2%
s	Tannerella forsythia	0,1%	0,1%	s	Streptococcus sp. oral taxon 058	0,2%	0,3%
s	Streptococcus sanguinis	0,1%	0,3%	s	Peptoclostridium yurii	0,2%	0,5%
g	Mogibacterium	0,1%	0,2%	s	Leptotrichia sp. oral taxon 498	0,2%	0,3%
s	Eubacterium saphenum	0,1%	0,3%	s	Tannerella forsythia	0,2%	0,1%
g	Bergeyella	0,1%	0,1%	s	Selenomonas sp. oral taxon 478	0,2%	0,5%
s	Prevotella oulorum	0,1%	0,1%	s	Eubacterium sp. oral taxon 081	0,2%	0,3%
s	Johnsonella sp. oral taxon 166	0,1%	0,0%	s	Filifactor alocis	0,2%	0,3%
s	Treponema sp. IV:17B:D4B	0,1%	0,1%	s	Dialister invisus	0,2%	0,4%
s	Cryptobacterium curtum	0,1%	0,1%	s	Selenomonas sputigena	0,2%	0,4%
g	Cardiobacterium	0,1%	0,1%	s	Actinomyces sp. oral taxon 171	0,2%	0,1%
s	Treponema maltophilum	0,1%	0,1%	s	Leptotrichia sp. oral taxon 462	0,2%	0,3%
s	Fusobacterium hwasookii	0,1%	0,2%	s	Atopobium sp. DMCT15023	0,1%	0,1%
s	Lautropia mirabilis	0,1%	0,0%	s	Prevotella micans	0,1%	0,2%

PATIENT F05				PATIENT F06			
Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected	Taxonomic Level	Taxonomic Unit	Fraction	Uncorrected
	Other	2,9%		s	Prevotella sp. oral taxon 314	0,1%	0,2%
	Unassigned	2441		s	Parasporobacterium paucivorans	0,1%	0,2%
	Filtered	0		s	Selenomonas dianae	0,1%	0,4%
				s	Prevotella sp. E9 42	0,1%	0,2%
				s	Treponema sp. IV:17B:D4B	0,1%	0,1%
				s	Bergeyella sp. oral taxon 322	0,1%	0,2%
				s	Streptococcus mitis	0,1%	0,3%
				s	Treponema sp. oral taxon 247	0,1%	0,1%
				s	Prevotella marshii	0,1%	0,1%
				s	Tannerella sp. oral taxon 808	0,1%	0,1%
				s	Eubacterium sp. oral strain A35MT	0,1%	0,2%
				s	Capnocytophaga sp. 'ARUP UnID 185'	0,1%	0,1%
				f	Flavobacteriaceae	0,1%	0,1%
				s	Prevotella maculosa	0,1%	0,1%
				s	Treponema medium	0,1%	0,1%
				s	Slackia exigua	0,1%	0,1%
				s	Parvimonas micra	0,1%	0,2%
				g	Cardiobacterium	0,1%	0,1%
				g	Brachymonas	0,1%	0,1%
				s	Fusobacterium sp. oral taxon A42	0,1%	0,2%
				s	Propionivibrio sp. oral taxon C33	0,1%	0,1%
				s	Porphyromonas sp. oral taxon 279	0,1%	0,1%
				s	Eubacterium sp. oral taxon G32	0,1%	0,2%
				s	Anaeroglobus geminatus	0,1%	0,3%
				s	Sporobacterium olearium	0,1%	0,1%
				s	Schwartzia sp. canine oral taxon 042	0,1%	0,3%
					Other	4,0%	
					Unassigned	4846	
					Filtered	0	

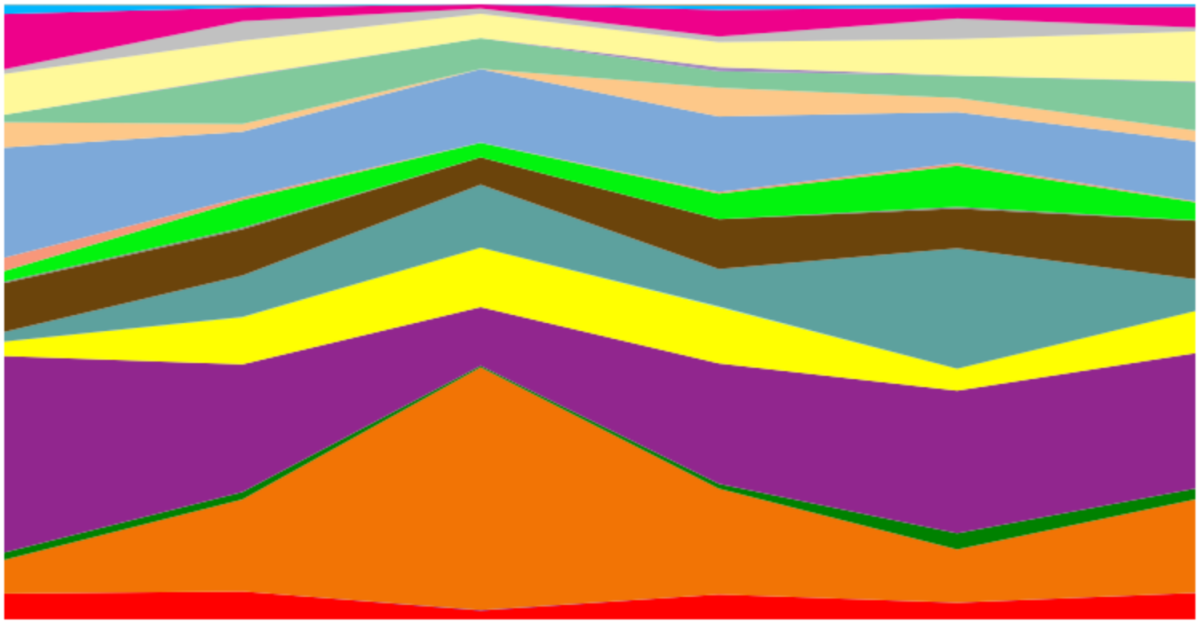
The following plots represent the different results for each sample in different level of taxonomy (Phylum, class and order because higher level plots give a more coarse grained view on the data than lower level plots).

PHYLUM



Legend	Taxonomy	Total count	Total %	F01 PD %	F02 PD %	F03 PD %	F04 PD %	F05 PD %	F06 PD %
	Unclassified;Other	10029	4%	4%	5%	1%	4%	3%	4%
	k__Bacteria;p__	55	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Actinobacteria	54308	19%	7%	16%	40%	18%	11%	17%
	k__Bacteria;p__Bacteroidetes	77926	28%	34%	28%	19%	29%	27%	29%
	k__Bacteria;p__Chloroflexi	77	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes	54846	19%	14%	19%	17%	19%	33%	18%
	k__Bacteria;p__Fusobacteria	33774	12%	18%	11%	12%	12%	8%	10%
	k__Bacteria;p__Proteobacteria	40847	14%	13%	18%	10%	13%	15%	19%
	k__Bacteria;p__Spirochaetes	9937	4%	9%	2%	1%	4%	2%	3%
	k__Bacteria;p__Synergistetes	1594	1%	2%	0%	0%	1%	1%	0%
	k__Bacteria;p__Tenericutes	248	0%	0%	0%	0%	0%	0%	0%

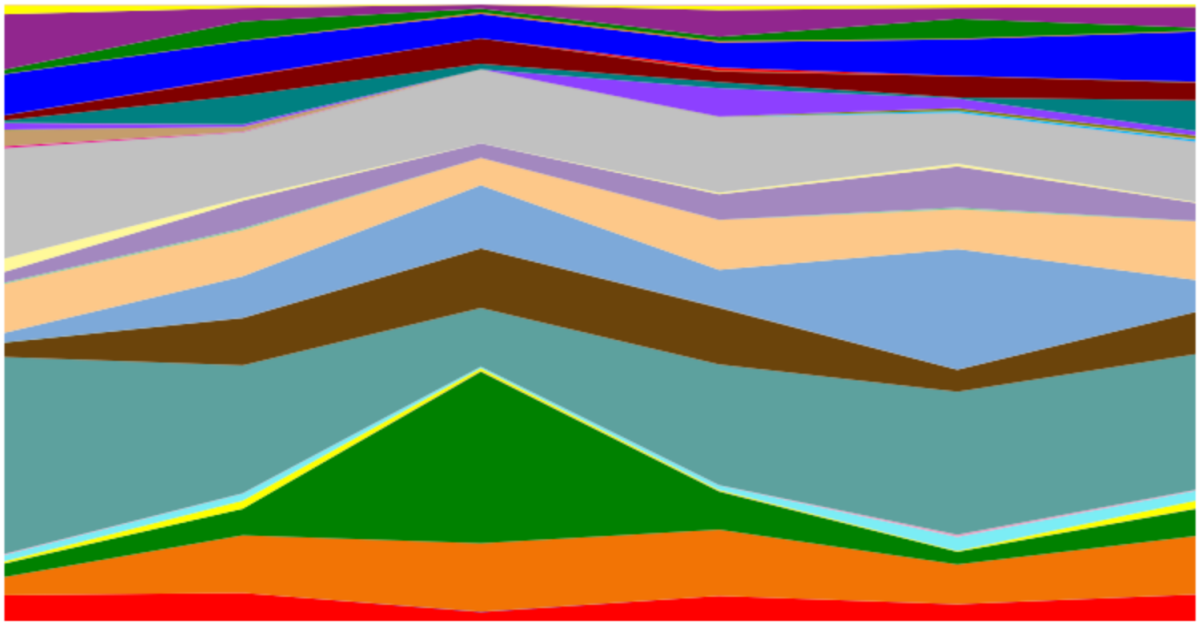
CLASS



Legend	Taxonomy	Total count	Total %	F01 PD %	F02 PD %	F03 PD %	F04 PD %	F05 PD %	F06 PD %
	Unclassified;Other;Other	10029	4%	4%	5%	1%	4%	3%	4%
	k__Bacteria;p__;c__	55	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria	50912	18%	6%	15%	40%	17%	9%	15%
	k__Bacteria;p__Actinobacteria;c__Coriobacteriia	3396	1%	1%	1%	0%	1%	3%	2%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia	58401	21%	32%	21%	10%	20%	23%	22%
	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia	19525	7%	2%	8%	10%	9%	4%	7%
	k__Bacteria;p__Chloroflexi;c__Anaerolineae	77	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__	9	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__Bacilli	22057	8%	2%	7%	10%	6%	20%	5%
	k__Bacteria;p__Firmicutes;c__Clostridia	20683	7%	8%	8%	4%	8%	6%	10%
	k__Bacteria;p__Firmicutes;c__Erysipelotrichia	377	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__Negativicutes	10161	4%	2%	5%	2%	4%	7%	3%
	k__Bacteria;p__Firmicutes;c__Tissierellia	1559	1%	2%	1%	0%	0%	1%	0%
	k__Bacteria;p__Fusobacteria;c__Fusobacteriia	33774	12%	18%	11%	12%	12%	8%	10%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	6594	2%	4%	1%	0%	5%	2%	2%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	13501	5%	1%	8%	5%	3%	4%	8%
	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	471	0%	0%	0%	0%	1%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria	15972	6%	7%	6%	4%	4%	6%	8%

	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	4309	2%	1%	3%	1%	1%	3%	1%
	k__Bacteria;p__Spirochaetes;c__Spirochaetia	9937	4%	9%	2%	1%	4%	2%	3%
	k__Bacteria;p__Synergistetes;c__Synergistia	1594	1%	2%	0%	0%	1%	1%	0%
	k__Bacteria;p__Tenericutes;c__Mollicutes	248	0%	0%	0%	0%	0%	0%	0%

ORDER

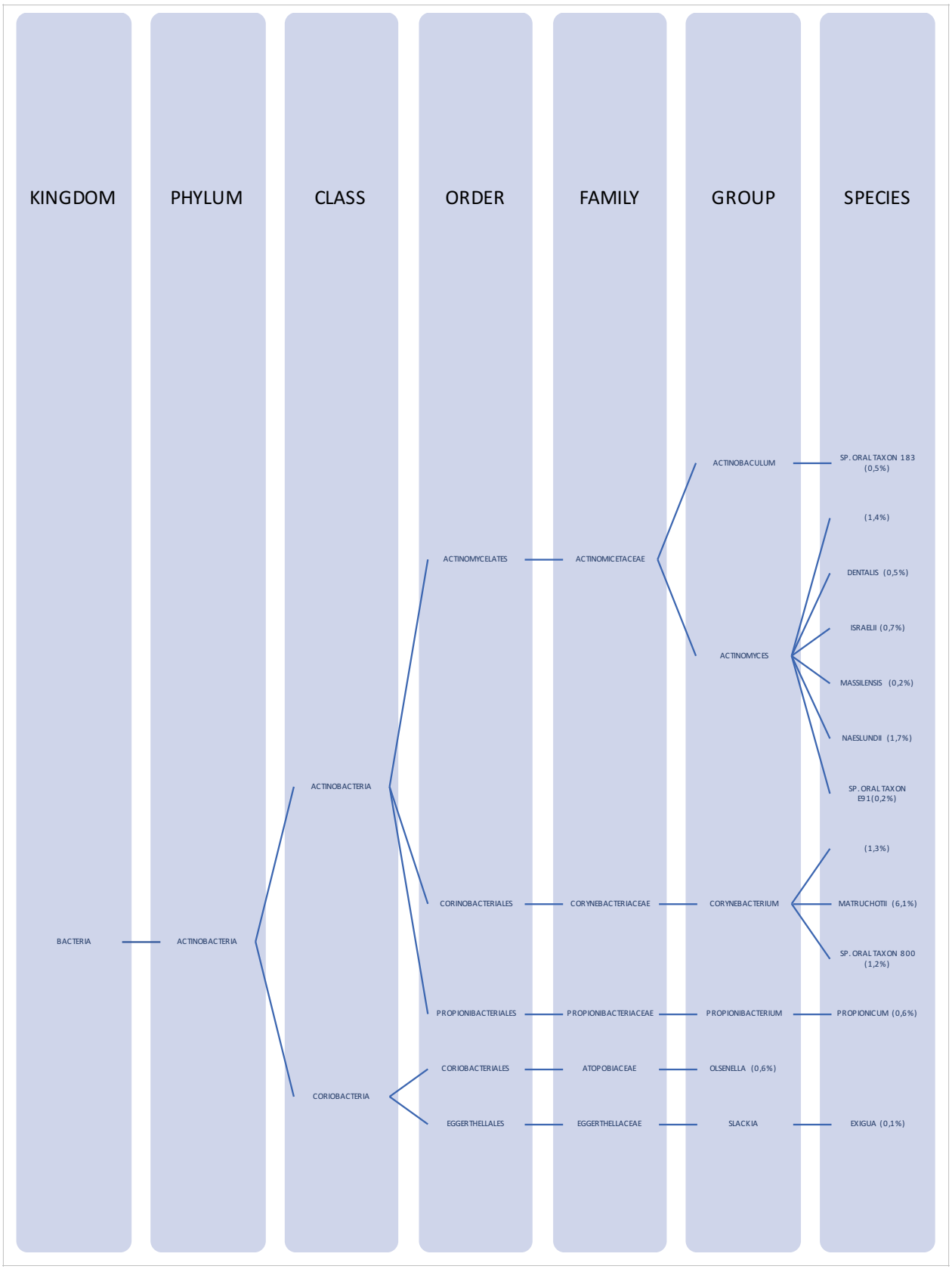


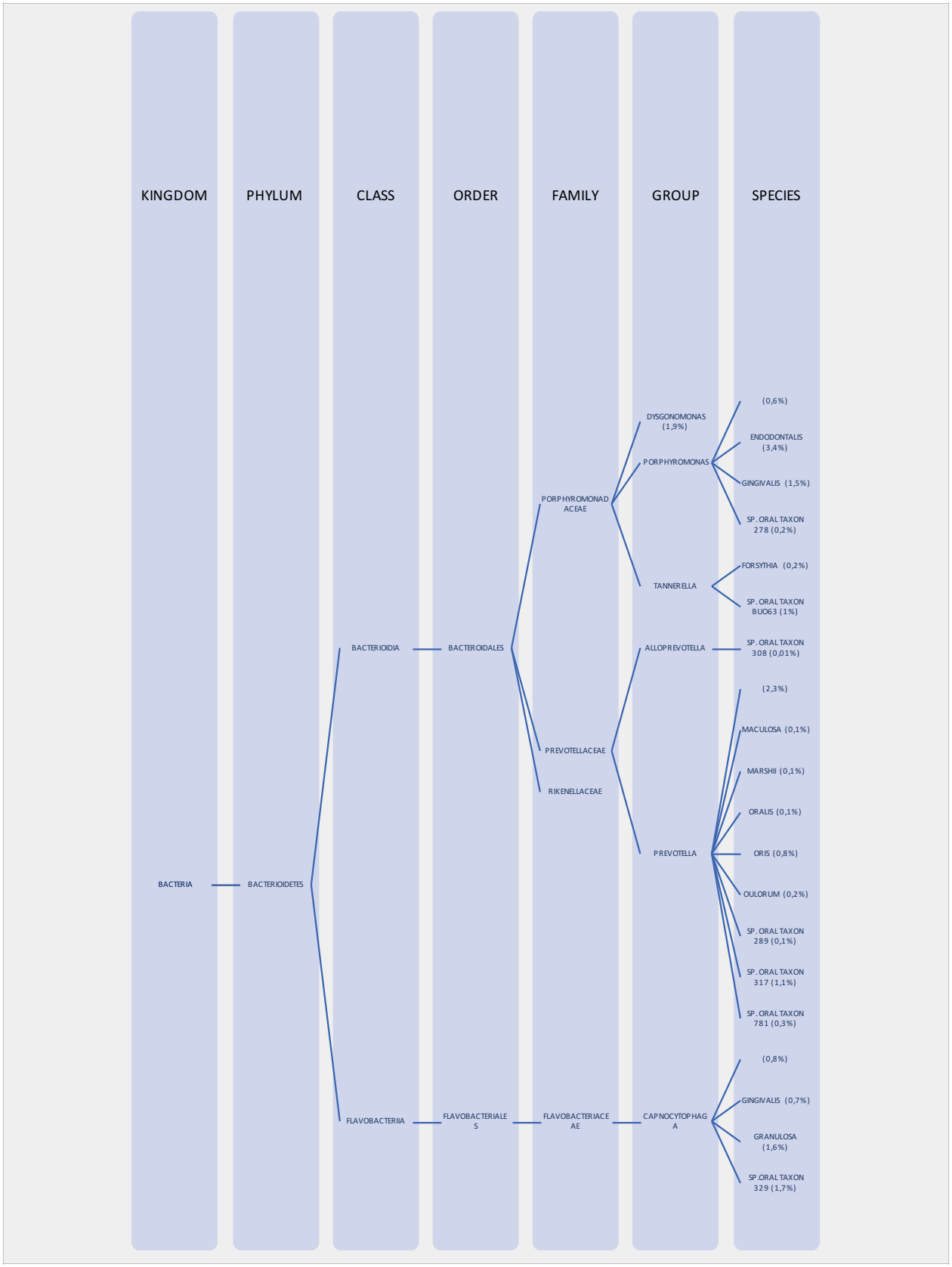
		Total		F01 PD	F02 PD	F03 PD	F04 PD	F05 PD	F06 PD
Legend	Taxonomy	count	%	%	%	%	%	%	%
	Unclassified;Other;Other;Other	10029	4%	4%	5%	1%	4%	3%	4%
	k__Bacteria;p__;c__;o__	55	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	24472	9%	3%	9%	11%	11%	6%	10%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Corynebacteriales	24493	9%	2%	4%	28%	6%	2%	4%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Micrococcales	124	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Propionibacteriales	1823	1%	0%	1%	1%	0%	0%	1%
	k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales	3098	1%	1%	1%	0%	1%	2%	2%
	k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Eggerthellales	298	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	58401	21%	32%	21%	10%	20%	23%	22%
	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales	19525	7%	2%	8%	10%	9%	4%	7%
	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales	77	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__;o__	9	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__	12	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales	22045	8%	2%	7%	10%	6%	20%	5%

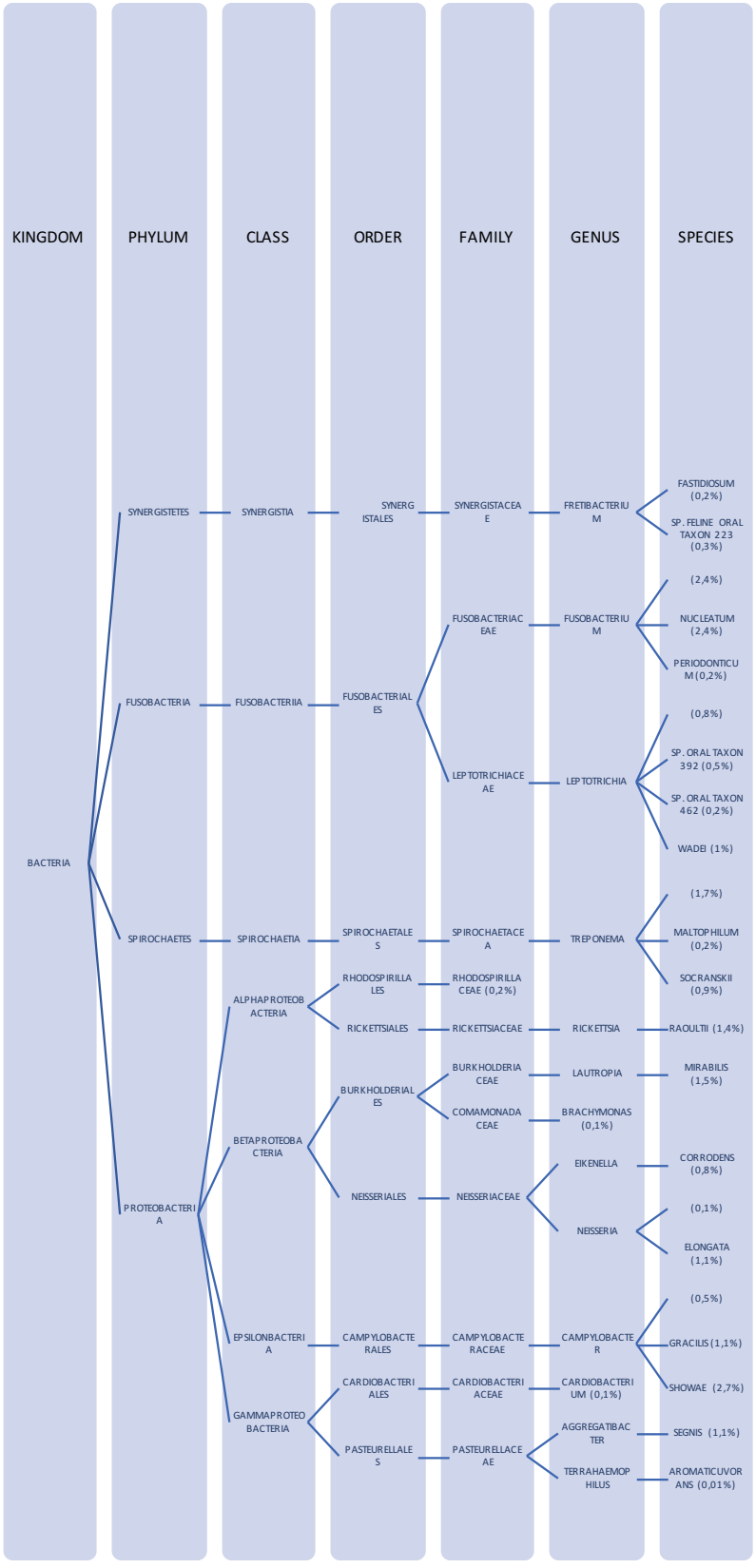
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	20683	7%	8%	8%	4%	8%	6%	10%
	k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales	377	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Firmicutes;c__Negativicutes;o__Selenomonadales	10161	4%	2%	5%	2%	4%	7%	3%
	k__Bacteria;p__Firmicutes;c__Tissierellia;o__Tissierellales	1559	1%	2%	1%	0%	0%	1%	0%
	k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales	33774	12%	18%	11%	12%	12%	8%	10%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__	186	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Magnetococcales	276	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	1569	1%	3%	1%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales	486	0%	0%	0%	0%	0%	0%	1%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales	4035	1%	1%	0%	0%	5%	2%	1%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sneathiellales	42	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	5896	2%	0%	5%	1%	1%	0%	5%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Neisseriales	7530	3%	1%	3%	4%	2%	4%	3%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales	75	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales	79	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales	392	0%	0%	0%	0%	1%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales	15972	6%	7%	6%	4%	4%	6%	8%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales	409	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales	3900	1%	1%	3%	1%	1%	3%	1%
	k__Bacteria;p__Spirochaetes;c__Spirochaetia;o__Spirochaetales	9937	4%	9%	2%	1%	4%	2%	3%
	k__Bacteria;p__Synergistetes;c__Synergistia;o__Synergistales	1594	1%	2%	0%	0%	1%	1%	0%
	k__Bacteria;p__Tenericutes;c__Mollicutes;o__Acholeplasmatales	56	0%	0%	0%	0%	0%	0%	0%
	k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales	192	0%	0%	0%	0%	0%	0%	0%

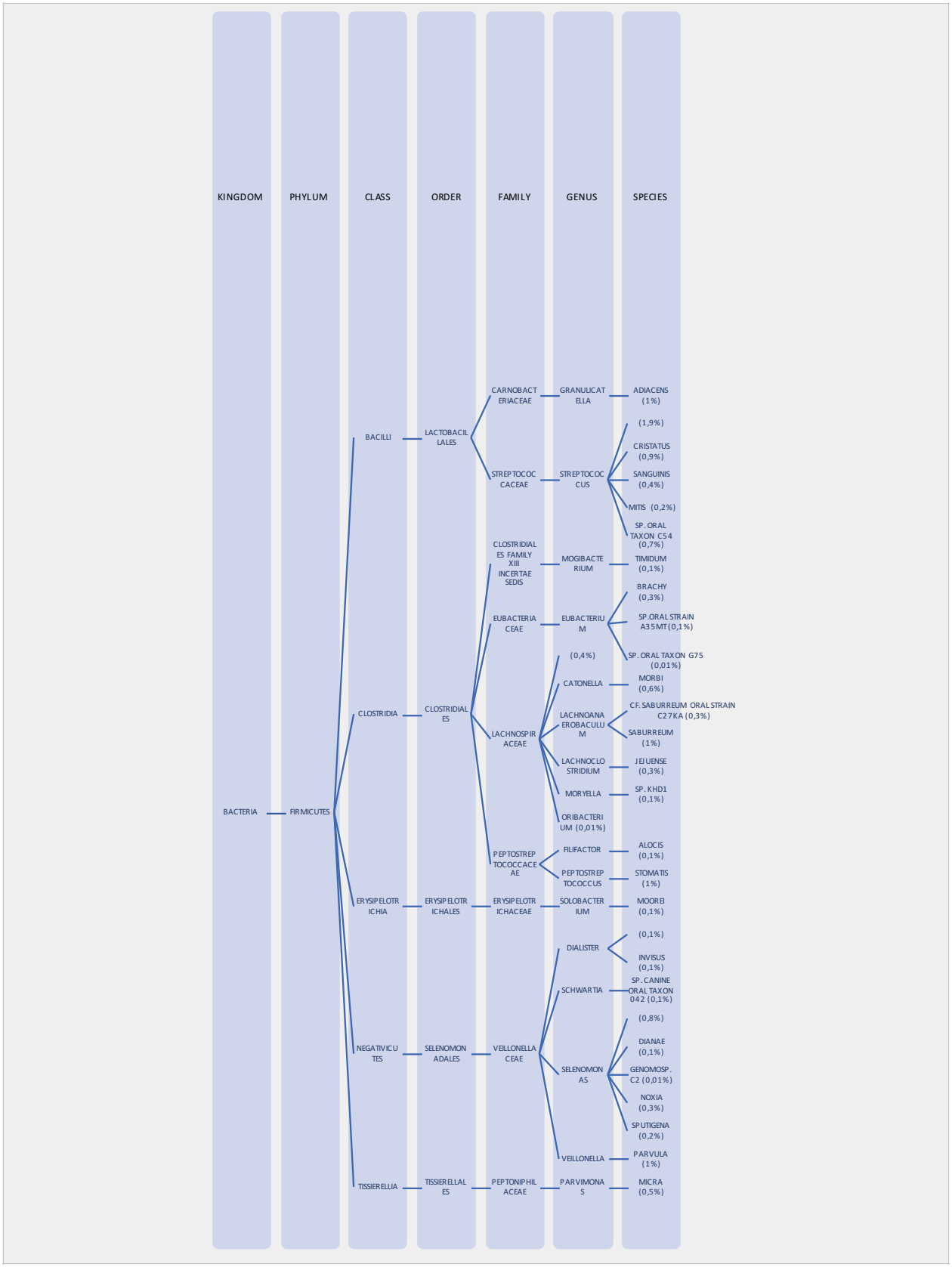
The carotid specimens of all 6 patients revealed evidence of severe atherosclerosis; the plaques showed a calcified core with frequent circumferential fatty deposit. Not enough DNA of periodontal bacteria was detected in any of the carotid samples using the same methods.

Comparing the oral microbiome we found a group of bacteria present in all patient showed in the following charts with percentage.


KINGDOM
PHYLUM
CLASS
ORDER
FAMILY
GROUP
SPECIES
BACTERIA
ACTINOBACTERIA
ACTINOBACTERIA
CORIOBACTERIA
ACTINOMYCELATES
CORINOBACTERIALES
PROPIONIBACTERIALES
CORIOBACTERIALES
EGGERTHELLALES
ACTINOMICETACEAE
CORYNEBACTERIACEAE
PROPIONIBACTERIACEAE
ATOPOBIACEAE
EGGERTHELLACEAE
ACTINOBACULUM
ACTINOMYCES
CORYNEBACTERIUM
PROPIONIBACTERIUM
OLSENELLA (0,6%)
SLACKIA
SP. ORAL TAXON 183 (0,5%)
(1,4%)
DENTALIS (0,5%)
ISRAELII (0,7%)
MASSILENSIS (0,2%)
NAESLUNDII (1,7%)
SP. ORAL TAXON E91(0,2%)
(1,3%)
MATRUCHOTII (6,1%)
SP. ORAL TAXON 800 (1,2%)
PROPIONICUM (0,6%)
EXIGUA (0,1%)


KINGDOM
PHYLUM
CLASS
ORDER
FAMILY
GROUP
SPECIES
BACTERIA
BACTERIOIDETES
BACTERIOIDIA
BACTEROIDALES
PORPHYROMONADACEAE
PREVOTELLACEAE
RIKENELLACEAE
DYSGONOMONAS (1,9%)
PORPHYROMONAS
TANNERELLA
ALLOPREVOTELLA
PREVOTELLA
(0,6%)
ENDODONTALIS (3,4%)
GINGIVALIS (1,5%)
SP. ORAL TAXON 278 (0,2%)
FORSYTHIA (0,2%)
SP. ORAL TAXON BUO63 (1%)
SP. ORAL TAXON 308 (0,01%)
(2,3%)
MACULOSA (0,1%)
MARSHII (0,1%)
ORALIS (0,1%)
ORIS (0,8%)
OULORUM (0,2%)
SP. ORAL TAXON 289 (0,1%)
SP. ORAL TAXON 317 (1,1%)
SP. ORAL TAXON 781 (0,3%)
FLAVOBACTERIIA
FLAVOBACTERIALES
FLAVOBACTERIACEAE
CAPNOCYTOPHAGA
(0,8%)
GINGIVALIS (0,7%)
GRANULOSA (1,6%)
SP. ORAL TAXON 329 (1,7%)


KINGDOM
PHYLUM
CLASS
ORDER
FAMILY
GENUS
SPECIES
BACTERIA
SYNERGISTETES
SYNERGISTIA
SYNERGISTALES
SYNERGISTACEAE
FRETIBACTERIUM
FASTIDIOSUM (0,2%)
SP. FELINE ORAL TAXON 223 (0,3%)
FUSOBACTERIA
FUSOBACTERIIA
FUSOBACTERIALES
FUSOBACTERIACEAE
FUSOBACTERIUM
(2,4%)
NUCLEATUM (2,4%)
PERIODONTICUM (0,2%)
LEPTOTRICHIACEAE
LEPTOTRICHIA
(0,8%)
SP. ORAL TAXON 392 (0,5%)
SP. ORAL TAXON 462 (0,2%)
WADEI (1%)
SPIROCHAETES
SPIROCHAETIA
SPIROCHAETALES
SPIROCHAETACEA
TREPONEMA
(1,7%)
MALTOPHILUM (0,2%)
SOCRANSKII (0,9%)
PROTEOBACTERIA
ALPHAPROTEOBACTERIA
RHODOSPIRILLALES
RHODOSPIRILLACEAE (0,2%)
RICKETTSIALES
RICKETTSIACEAE
RICKETTSIA
RAOULTII (1,4%)
BETAPROTEOBACTERIA
BURKHOLDERIALES
BURKHOLDERIACEAE
LAUTROPIA
MIRABILIS (1,5%)
COMAMONADACEAE
BRACHYMONAS (0,1%)
NEISSERIALES
NEISSERIACEAE
EIKENELLA
CORRODENS (0,8%)
NEISSERIA
(0,1%)
ELONGATA (1,1%)
EPSILONBACTERIA
CAMPYLOBACTERALES
CAMPYLOBACTERACEAE
CAMPYLOBACTER
(0,5%)
GRACILIS (1,1%)
SHOWAE (2,7%)
GAMMAPROTEOBACTERIA
CARDIOBACTERIALES
CARDIOBACTERIACEAE
CARDIOBACTERIUM (0,1%)
PASTEURELLALES
PASTEURELLACEAE
AGGREGATIBACTER
SEGNIS (1,1%)
TERRAHAEMOPHILUS
AROMATICUVORANS (0,01%)


KINGDOM
PHYLUM
CLASS
ORDER
FAMILY
GENUS
SPECIES
BACTERIA
FIRMICUTES
BACILLI
LACTOBACILLALES
CARNOBACTERIACEAE
GRANULICATELLA
ADIACENS (1%)
STREPTOCOCCACEAE
STREPTOCOCCUS
(1,9%)
CRISTATUS (0,9%)
SANGUINIS (0,4%)
MITIS (0,2%)
SP. ORAL TAXON C54 (0,7%)
CLOSTRIDIA
CLOSTRIDIALES
CLOSTRIDIALES FAMILY XIII INCERTAE SEDIS
MOGIBACTERIUM
TIMIDUM (0,1%)
EUBACTERIACEAE
EUBACTERIUM
BRACHY (0,3%)
SP.ORAL STRAIN A35MT(0,1%)
SP. ORAL TAXON G75 (0,01%)
LACHNOSPIRACEAE
(0,4%)
CATONELLA
MORBI (0,6%)
LACHNOANAEROBACULUM
CF. SABURREUM ORAL STRAIN C27KA (0,3%)
SABURREUM (1%)
LACHNOCLOSTRIDIUM
JEJUENSE (0,3%)
MORYELLA
SP. KHD1 (0,1%)
ORIBACTERIUM (0,01%)
PEPTOSTREPTOCOCCACEAE
FILIFACTOR
ALOCIS (0,1%)
PEPTOSTREPTOCOCCUS
STOMATIS (1%)
ERYSIPELOTRICHIA
ERYSIPELOTRICHALES
ERYSIPELOTRICHACEAE
SOLOBACTERIUM
MOOREI (0,1%)
NEGATIVICUTES
SELENOMONADALES
VEILLONELLACEAE
DIALISTER
(0,1%)
INVISUS (0,1%)
SCHWARTIA
SP. CANINE ORAL TAXON 042 (0,1%)
SELENOMONAS
(0,8%)
DIANAE (0,1%)
GENOMOSP. C2 (0,01%)
NOXIA (0,3%)
SPUTIGENA (0,2%)
VEILLONELLA
PARVULA (1%)
TISSIERELLIA
TISSIERELLALES
PEPTONIPHILACEAE
PARVIMONAS
MICRA (0,5%)

2.3 DISCUSSION

Different studies have suggested a possible association between periodontitis and the extent and severity of cardiovascular disease[29, 33-35]. However, complete evidence has not been established in this field and other studies which questioned this association have been published[80-82]. Experimental studies have demonstrated the ability of such periodontal pathogens to interact with the endothelial surface and to induce smooth cell proliferation and the local release of inflammatory cytokines. Therefore the presence of periodontal bacteria in human atherosclerotic plaques may play a role in the initiation, development and progression stage of atherosclerosis.

The present study was first aimed to ascertain the presence of periodontal bacteria DNA in carotid atheroma in dentate patients. A total of 6 carotid samples were collected and analyzed for 16S Microbiome profiling (region V1-V3) but in none of the atherosclerotic plaque samples there was enough DNA of periodontal pathogen detection. We have to remember that in our population none of the patient had severe periodontitis.

Our data agree with Cairo et al[83] but do not concur with the results published by Chiu[56] (immunocytochemical investigation; 42% of carotid plaques positive for P Gingivalis), Haraszthy et al[17] (PCR amplified 16S rDNA and DNA species-species probes, 30% positive for T. Forsythensis, 26% for P. Gingivalis, 18% A Actinomycetemcomitans, 14% for P. Intermedia), Taylor-Robinson et al[84] (PCR-amplified 16S rDNA and universal primers, 22% positive for A Actinomycetemcomitans and 9% for P. Intermedia) and Ishihara et al[57] (PCR-amplified 16S rRNA, 21% positive for P. Gingivalis, 23% for A. Actinomycetemcomitans, 5,9% for T. Forsythensis). Such differences among various research works indicate that microbial associations in atherosclerotic plaques are highly diverse and variable between individual patients.

These studies identify at least one, but often multiple, periodontal pathogen in atheromatous samples, whereas we have no evidence of periodontal bacteria DNA in our sample collection. A possible reason for this difference could be the methodology used in different laboratories. An alternative hypothesis, even if less probable, could be that the prevalence of periodontal bacteria DNA in atheromatous lesions differs due to epidemiological reasons (disease stage,

nutrition, geographic factors, ethnicity…). However, the presence of bacterial DNA in atheromatous plaques still remains a controversial issue.

The cumulative evidence in the literature supports, but does not prove, a causal association between periodontal infection and atherosclerotic cardiovascular disease or its sequelae. A number of legitimate concerns have arisen about the nature of this relationship and, indeed, about the appropriate definitions for periodontal disease when is thought to be an exposure for systemic disease.

Periodontal pathogens have been shown to contribute directly to atherosclerosis by disrupting endothelial cell function, one of the earliest indicators of cardiovascular disease. Oral infection is thought to indirectly induce elevated production of inflammatory mediators in the systemic circulation and it is clear that the immune mechanisms leading to atherosclerotic plaque progression, by oral infection, are complex[25]. Understanding the immune pathways leading to disease progression is essential for the future development of anti-inflammatory therapies for this chronic disease. Indeed chronic extravasal inflammatory processes may also interact with atherosclerotic lesions. For example, is known that patients suffering from chronic infections have 4.1 times higher risk to develop carotid atherosclerosis compared to none[85]. The routes on how local infections induce systemic effects have been studied extensively in recent years, since it became evident that local infections may contribute to the initiation and progression of several clinical diseases in humans. The relevant pathways of action are by direct migration of bacteria from local biofilms to tissues and organs using the bloodstream or lymphatic vessels, or by the local release of inflammatory mediators and pathogenic agents into the bloodstream to exert inflammatory reactions in predisposed tissues or organs.

In conclusion several studies were published that reported the identification of periopathogens in atherosclerotic plaques for uncovering the pathological pathways between periodontal and atherosclerotic diseases. Bacteria that have been associated with carious lesions were found in atherosclerotic plaques as well. However, several studies, as ours, were not able to find periopathogens in atherosclerotic plaques, and hence, the transmission of bacteria from the oral cavity to atherosclerotic plaques and their direct effects are still under debate[83, 86].

3. CONCLUSIONS

The critical question of whether periodontal infections are a risk factor for or contribute casually to cardiovascular disease remains unanswered. The possibility that periodontal disease and cardiovascular disease share common risk factors or are manifestations of a similar underlying pathology remains. As mentioned periodontal and cardiovascular disease share common risk factors so a correlation between the two would be expected even if a causal link did not exist.

However, at this point of time, it is difficult to pin point whether it is the stand-alone role of individual periodontal pathogen or the total periodontal border as a whole, which contribute towards atherosclerosis. Further molecular studies are required to elucidate the role of periodontitis inflammation for a better understanding of this association.

There is, however, no direct peer-reviewed evidence to suggest that treating or preventing periodontal infections leads to fewer clinical cardiovascular events. Recommending periodontal treatment solely for the purpose of atherosclerotic cardiovascular disease prevention is not warranted based on current scientific evidence. Periodontal treatment must be recommended on the basis of the value of its benefits for the oral health of patients, recognizing that patients are not healthy without good oral health and taking into account American Heart Association recommendations[87].

It is important also to remember that atherosclerosis has a strong inflammatory component and epidemiological evidence suggests that increased levels of systemic inflammation are predictive of cardiovascular events and is almost certain that people with periodontal disease have elevated levels of systemic inflammatory markers such as C-Reactive Protein. Triggers for this enhanced systemic inflammatory response include transient bacteremia and local release of bacteria byproducts.

In conclusion, the results of this study do not support the previous findings that reported a frequent presence of periodontal pathogens in carotid atheroma lesions. Our data, therefore, tend to exclude a direct correlation between the detection of periodontal bacteria DNA in oral lesions and its concomitant presence in carotid atheroma. However, the current evidence supporting association raises an important question: “If periodontal infection is suppressed by anti-infective intervention, will this result in a decreased risk of cardiovascular disease?” Answers to this questions would be clinically meaningful and may more directly implicate

periodontal disease as risk factor for cardiovascular disease for his systemic effects. Some studies have evaluated the impact of periodontal treatment, with or without antimicrobial therapy, on systemic inflammation or endothelial dysfunction and have shown mixed results[88].

About our study it is necessary to extend the number of cases to obtain well-founded result. But the direction of future research must be primarily to understand mechanisms that can relate periodontitis to atherosclerosis, in particular regarding inflammatory cascade.

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