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► **To cite this version:**

Steeve Bregaint, Emile Boyer, Shao Bing Fong, Vincent Meuric, Martine Bonnaure-Mallet, et al.. Porphyromonas gingivalis outside the oral cavity. *Odontology*, 2022, 110 (1), pp.1-19. 10.1007/s10266-021-00647-8 . hal-03331729

**HAL Id: hal-03331729**

**<https://hal.science/hal-03331729>**

Submitted on 16 Sep 2021

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*Porphyromonas gingivalis* outside the oral cavity

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28 **ABSTRACT**

29 *Porphyromonas gingivalis*, a Gram-negative anaerobic bacillus present in periodontal disease, is considered one of  
30 the major pathogens in periodontitis. A literature search for English original studies, case series and review articles  
31 published up to December 2019 was performed using the MEDLINE, PubMed and GoogleScholar databases, with  
32 the search terms "*Porphyromonas gingivalis*" AND the potentially associated condition or systemic disease  
33 Abstracts and full text articles were used to make a review of published research literature on *P. gingivalis* outside  
34 the oral cavity. The main points of interest of this narrative review were: (i) a potential direct action of the bacterium  
35 and not the systemic effects of the inflammatory acute-phase response induced by the periodontitis, (ii) the presence  
36 of the bacterium (viable or not) in the organ, or (iii) the presence of its virulence factors.

37 Virulence factors (gingipains, capsule, fimbriae, hemagglutinins, lipopolysaccharide, hemolysin, iron uptake  
38 transporters, toxic outer membrane blebs/vesicles, and DNA) associated with *P. gingivalis* can deregulate certain  
39 functions in humans, particularly host immune systems, and cause various local and systemic pathologies. The most  
40 recent studies linking *P. gingivalis* to systemic diseases were discussed, remembering particularly the molecular  
41 mechanisms involved in different infections, including cerebral, cardiovascular, pulmonary, bone, digestive and  
42 peri-natal infections. Recent involvement of *P. gingivalis* in neurological diseases has been demonstrated. *P.*  
43 *gingivalis* modulates cellular homeostasis and increases markers of inflammation. It is also a factor in the oxidative  
44 stress involved in beta-amyloid production.

45

46 **KEY WORDS:** *Porphyromonas gingivalis*; systemic disease; virulence factor; gingipain; brain; bone;  
47 cardiovascular; lung.

48

49 **1 INTRODUCTION**

50 *Porphyromonas gingivalis* (*P. gingivalis*) is a strictly anaerobic bacterium and commonly associated with  
51 a chronic pathology of the gums: gingivitis or periodontitis, oral inflammation which destroys the gum and the bones  
52 carrying the teeth. An updated classification of periodontal diseases and a new classification of peri-implant diseases  
53 and conditions have been discussed by Caton *et al.* [1], following the work of the worldwide community of scholars  
54 and clinicians in periodontology and implant dentistry [1]. If periodontitis is not treated, periodontal pockets develop  
55 between the gum and the tooth, the inflammation spreading causing other complications such as halitosis, caused by  
56 synthesis of many substances present in the oral cavity and metabolized by *P. gingivalis* allowing the synthesis of  
57 volatile, malodorous sulfur compounds [2]. The involvement of *P. gingivalis* in pathologies other than periodontal  
58 disease has been suspected.

59 *P. gingivalis* has multiple virulence factors that may be responsible for the severity of disease following a  
60 mixed infection [3]. Virulence factors include capsule proteins [4,5], gingipains (proteinases) [6,7], fimbriae [8-11],  
61 hemagglutinins [12-14], lipopolysaccharide (LPS) [15-18], hemolysin, iron uptake transporters, toxic outer  
62 membrane blebs/vesicles, and DNA. Production of both gingipains and fimbriae are mechanistically important for  
63 the dysregulation of immune function, and the expression of capsule proteins aids in the evasion of immune effectors  
64 [19,20]. Phosphatidylinositol 3-kinase (PI3K) and Akt are well known to play a pivotal role in various cellular  
65 physiological functions including cell survival and glucose metabolism in mammalian cells [21]. These virulence  
66 factors participate in the pathogenicity of bacteria in infected tissues and enable bacterial dissemination. *In vitro* and  
67 clinical studies have shown *P. gingivalis* invasion and survival in non-oral human cells, such as coronary arterial  
68 endothelial and placental cells, where they could potentially contribute to localized inflammatory responses [22,23].  
69 Lysine gingipain (Kgp), arginine gingipain A (Rgp A) and arginine gingipain B (Rgp B) are a set of cysteine  
70 proteinases. These enzymes play several roles in the cleavage and activation of numerous host and bacterial proteins.  
71 The first role is to capture nutrients (heme, peptides, and amino acids) through hydrolysis [24,25]. Other roles include  
72 aggregation (for example, with hemagglutinin domains in biofilm), cell adhesion, and cell invasion [26,27].  
73 Gingipains also act to dysregulate the host's innate and adaptive immune responses: gingipains can cleave cell  
74 surface receptors, cytokines, and antibodies (IgG, IgM, and C3 complement protein) [28]. Liu *et al.* [29] provided  
75 the first evidence that Rgp and Kgp cooperatively contribute to *P. gingivalis*-induced cell migration and the  
76 expression of pro-inflammatory mediators. Finally, as part of the C-terminal domain protein family, gingipains are  
77 more numerous on vesicle surfaces [30]. Vesicles [30-32], evaginations of the outer cell membrane measuring 50 to  
78 300 nm, are formed by a membrane bilayer composed of A-LPS and proteins with a C-terminal domain (CTD), such

79 as gingipains, peptidyl deaminase (PAD), or hemin-binding protein 35 (HBP35). Fimbriae allow bacteria to adhere  
80 to prokaryotic host cells, extramolecular matrix molecules, cytokines, and fibrinogens. As a pathogen-associated  
81 molecular pattern (PAMP), fimbriae can also bind to CD14,  $\beta$ 2 integrins, TLR2, and TLR4. These interactions cause  
82 the synthesis of pro-inflammatory cytokines (monocyte chemoattractant protein-1 (MCP-1), TNF- $\alpha$ , interleukin  
83 1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-8) and induce expression of a variety of molecular adhesives (intercellular adhesion  
84 molecule-1 (ICAM 1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin, E-selectin, CD40, CD80, and  
85 CD86) [33,34]. IL-8 is able to induce hemoglobin receptor (HbR), which binds hemoglobin and acts as hemophore  
86 to capture porphyrin and heme in need of iron for the growth of *P. gingivalis* [21].

87 Immune response, DNA methylation, post-translational modifications of DNA-associated histone protein  
88 in chromatin, and the activity of non-coding RNAs are regulated by epigenetic alterations [35]. The LPS of *P.*  
89 *gingivalis* is able to reduce DNA methylases and increase the levels of histone acetylase and NF- $\kappa$ B in human oral  
90 cells [36-38]. *P. gingivalis* can also produce short-chain fatty acids (SCFAs); higher levels of SCFAs have been  
91 detected in the saliva of patients with severe periodontal disease. SCFAs, such as butyric acid, were demonstrated to  
92 inhibit class-1/2 histone deacetylases and downregulate expression of histone N-lysine methyltransferases, which  
93 leads to gene expression by facilitating the binding of transcription factors and RNA polymerase to DNA strands  
94 [39,40]. This was confirmed by the use of DNA methylase inhibitors prior to *P. gingivalis* exposure [41], suggesting  
95 that DNA methylation differentially affects gingival cytokine secretion in response to *P. gingivalis*. *P. gingivalis* is  
96 therefore able to regulate gene expression, in particular virulence or latency-inducing genes, in other microorganisms  
97 such as viruses [42] or other bacterial species [43]. *P. gingivalis*-LPS was also demonstrated to induce production  
98 of beta-amyloid proteins (A $\beta$ 40 and A $\beta$ 42) in neural cell cultures, and strongly enhanced TNF- $\alpha$  and IL-1 $\beta$   
99 production in a culture of microglial cells primed with A $\beta$  [44].

100 Enzymes from *P. gingivalis* are transported through the periplasm before being exposed to the bacterial  
101 outer membrane, or released in an extracellular environment. Non-proteolytic enzymes of *P. gingivalis* include  
102 phospholipase A, alkaline phosphatase, acid phosphatase, nuclease, hyaluronidase, chondroitin sulfatase, and  
103 heparinase. *P. gingivalis* also produces a number of proteolytic enzymes, including serine proteinases, collagenases,  
104 and the trypsin-like cysteine proteinases. As part of its amino acid catabolism, *P. gingivalis* produces toxic  
105 compounds, such as volatile sulfur compounds, short chain fatty acids (butyrate, propionate), indole, and ammonia,  
106 which are toxic to host cells or play a role in immune modulation. Other compounds, such as phosphorylated  
107 dihydroceramide (PDHC) lipids produced by various intestinal and oral microorganisms including *P. gingivalis*,  
108 have been reported not only in gingival tissues, but also in blood, vascular tissues, and brain. These lipids have been

109 shown to induce cellular effects through TLR2, inducing the secretion of IL-6 by dendritic cells, which can affect  
110 systemic immune homeostasis [45].

111 However, bacteria that require anaerobic conditions (80% N<sub>2</sub>, 10% H<sub>2</sub>, 10% CO<sub>2</sub>) [46,47] are usually  
112 cultured on blood agar or Columbia agar supplemented with hemin, nutrients (tryptic soy, peptone/proteose, yeast),  
113 and vitamin K3 (menadione); most strains are cultured for at least 24 to 48 hours at 37°C [48,49]. Selective media  
114 can be used to isolate *P. gingivalis* from biological samples, such as antibiotics (colistin, bacitracin, nalidixic acid),  
115 essentially for research purposes [50]. Metabolic activities are not adapted to diagnosis for several reasons. First,  
116 they are time-consuming and influenced by culture conditions, medium composition, growth phase of each  
117 microorganism, and possible presence of viable but non-cultivable bacteria. The second reason is the limited number  
118 of classification criteria [51]. The use of microbial culturomics is re-emerging as one of the most reliable means of  
119 bacterial identification [52].

120 Many immunological techniques, such as indirect fluorescent antibody techniques [53] and enzyme-linked  
121 immunosorbent assay (ELISAs) [54,55], have been tested in the identification of suspected *P. gingivalis* in  
122 pathologies outside the oral cavity. These simple, quick, and very specific techniques are limited by the small number  
123 of available antibodies, and are therefore not adapted to polymicrobial samples.

124 Molecular diagnostic techniques can also be used, including molecular hybridization [56], real-time PCR  
125 (RT-PCR) [57] and multiplex PCR [58]. These methods, considered first-generation sequencing since Sanger *et al.*  
126 [59] and Maxam *et al.* [60], do not require cultivation. They are able to identify non-cultivable bacteria, but need  
127 specific probes for genome-sequenced bacteria, and require that the microorganisms studied have been sequenced  
128 beforehand.

129 Finally, metagenomics using new generation sequencing (NGS) or high-throughput sequencing (HTS)  
130 appeared in 2005 [46,61-64]. Similar to molecular diagnosis, cell culture is not needed. After total DNA has been  
131 extracted from the sample, hypervariable regions of the 16S rRNA gene are amplified using universal primers, then  
132 sequenced through the NGS process [65]. Sequence comparison with ribosomal databases (e.g., GenBank, EMBL,  
133 HOMO, RDP) is used to identify or evaluate phylogenetic distances between species. This method has highlighted  
134 the gaps of previous methods and allowed the reassessment of bacterial phenotyping.

135 Many oral bacteria also produce many putative virulence factors that would likely have similar effects to  
136 the ones described to *P. gingivalis*. However, many of these pathogens have not been studied in any detail and, in  
137 addition to studying the bacterial genome, NGS can identify all bacteria in the sample, even difficult to cultivate or  
138 non-cultivable [20]. Although NGS does have some limits (it does not allow for susceptibility testing of

139 antimicrobial agents, and it can also lead to errors, such as wrongly reporting new bacterial species), this technique  
140 may be useful for determining the presence of *P. gingivalis* outside periodontal tissues. Metagenomic and  
141 metabolomic analyzes will make it possible to highlight the potential cooperation between species. This review takes  
142 stock of the particular virulence of *P. gingivalis* and discusses microbiota studies related to associated systemic  
143 pathologies, by collecting the most recent studies linking *P. gingivalis* to systemic diseases. Implications of *P.*  
144 *gingivalis* infections in different systems are discussed, especially cerebral, cardiovascular, pulmonary, bone,  
145 digestive, and peri-natal systems.

146 A literature search for English original studies, case series and review articles published up to December  
147 2019 was performed using the MEDLINE, PubMed and GoogleScholar databases, with the search terms  
148 "*Porphyromonas gingivalis*" AND the potentially associated condition or systemic disease (i.e. "atherosclerosis",  
149 "diabetes mellitus", "cardiovascular disease", "cancer", "rheumatoid arthritis", "inflammatory bowel disease",  
150 "neurological disease", "chronic obstructive pulmonary disease"). Reference lists of relevant articles were  
151 additionally screened to reduce the risk of missing relevant information and to guide the search for potential  
152 association. The search terms "oral bacteria" and "periodontitis" were also used as alternatives to "*Porphyromonas*  
153 *gingivalis*", however, only articles explicitly mentioning the bacterium and reporting its potential activity in the  
154 organ of interest were retained. The main points of interest were: (i) a potential direct action of the bacterium and  
155 not the systemic effects of the inflammatory acute-phase response induced by the periodontitis, (ii) the presence of  
156 the bacterium (viable or not) in the organ, or (iii) the presence of its virulence factors.

157

## 158 2 *P. GINGIVALIS* AND DISEASES

159

### 160 2.1 *P. gingivalis* and brain diseases (Figure 1)

161 The relationship between infection of the oral cavity and systemic disease has been described often. In  
162 particular, the spread of infection from the oral cavity to the brain can lead to neurological diseases [66-69]. This  
163 was illustrated by clinical case reports describing brain abscesses in patients with recurrent periodontitis [70,71].

164 The detection of *P. gingivalis*-specific IgG intrathecal antibodies in early-stage Alzheimer's disease (AD)  
165 was associated with T-tau level [72]. Antibodies found in the serum of periodontitis patients have been correlated  
166 with a higher risk of developing neurological diseases such as AD or complicated Marfan syndrome [73,74]. Anti-  
167 *P. gingivalis* antibody levels were compared between cognitively intact control subjects and clinical participants

168 (diagnosed with AD or mild-cognitive impairment) at baseline blood draw and after cognitive conversion. *P.*  
169 *gingivalis*-specific antibodies were detected at significantly higher levels in patients who converted to AD, at  
170 baseline draw (when still cognitively normal). Interestingly, the antibody level measured in AD patients at baseline  
171 was similar to that of patients with chronic periodontitis. Due to the existence of *P. gingivalis*-specific antibodies in  
172 AD patients prior to their AD diagnosis, it was hypothesized that the elevation of these antibodies was not a result  
173 of secondary AD developmental factors, thus suggesting an association between periodontal pathogens and the  
174 development of AD [73].

175 In AD, *P. gingivalis* may affect permeability of the blood-brain barrier and inhibit local interferon- $\gamma$   
176 response by preventing the entry of immune cells into the brain [75]. Rokad *et al.* [76] revealed that the hippocampal  
177 microvascular structure of *P. gingivalis*-infected ApoE<sup>-/-</sup> mice were subjected to elevated oxidative stress levels,  
178 leading to the loss of functional integrity of tight junction proteins. Chronic local inflammation, observed in the case  
179 of periodontitis, leads to the release of inflammatory mediators, bacteria and/or their virulence factors, which can  
180 reach the brain *via* systemic circulation. This can induce a loss of neuronal integrity and loss of cellular function  
181 (memory alteration) [66,77]. The effects of periodontitis on organs such as the brain in terms of metabolic alterations  
182 remain unknown. Iliveski *et al.* [78] induced oral infection in mice by inoculating them with *P. gingivalis* and first  
183 demonstrated changes in redox homeostasis, with increases in markers of inflammation and oxidative stress and  
184 further amyloid beta production [79]. Dominy *et al.* [80] showed that gingipain can induce the bacterial load of an  
185 established *P. gingivalis* brain infection in a mice model, with levels correlated with tau and ubiquitin pathology,  
186 and stimulating A $\beta$ <sub>1-42</sub> (a component of amyloid plaques) production and neuroinflammation. Neuro-inflammation,  
187 strongly implicated in AD, can be enhanced during periodontitis through the intermediary of leptomenigeal cells,  
188 which are able to transduce pro-inflammatory signals from macrophages to microglia [81,82]. Furthermore, this  
189 neuro-inflammation is increased with aging, because senescent-type microglia are more susceptible to inflammatory  
190 processes [82]. The expression of TLR2, TLR4, TNF-alpha and inducible nitric oxide synthase (iNOS) are thought  
191 to be induced by LPS from *P. gingivalis* in M1 macrophages of chronic periodontitis patients [81]. However, when  
192 BALB/c mice were inoculated with *P. gingivalis*, Nemec *et al.* [83] observed lower levels of nitrite and nitric oxide  
193 in plasma and in the brain, respectively, compared with controls. Nitric oxide (NO) is involved in host defense  
194 mechanisms, and reduction of NO levels is likely to delay the host response, thereby promoting infection with *P.*  
195 *gingivalis*. However, Hayashi *et al.* [84] recently showed in a mouse model that continuous brain exposure of Pg-  
196 LPS started sarcopenia and cardiac injury without enhancing cognitive impairment in AD model mice.



197 Using anti-*P. gingivalis* specific antibodies, LPS from *P. gingivalis* has been detected in brain tissues from  
198 brain dementia patients, post-mortem, as well as on the surface membrane of the astrocyte cell line SVGp12 exposed  
199 to *P. gingivalis* LPS [85]. DNA from oral bacteria was detected in 70% of cases of ruptured and unruptured aneurysm  
200 samples in a recent study, and prevalence of *P. gingivalis* was higher in ruptured aneurysm [86]. In a 2015 study,  
201 Poole *et al.* [87] reported detection of bacterial DNA and complement activation (activated C3 fragments) in the  
202 brains of ApoE<sup>-/-</sup> mice that had been orally infected previously with *P. gingivalis*. Therefore, they suggested that *P.*  
203 *gingivalis* LPS was able to access the brain during AD manifestation and contributed to neuron injury [87].  
204 Administration of *P. gingivalis*-LPS can lead to learning and memory impairment in C57BL/6 mice, mediated by  
205 activation of the TLR4 signaling pathway [88]. Singhrao & Olsen [89] have highlighted the role of microvesicles in  
206 the transport of virulence factors, such as LPS, gingipains, capsule proteins, and fimbriae, secreted by *P. gingivalis*  
207 in bacterial cultures and established oral biofilms. Recently, Dominy *et al.* [80] even suggested that gingipain  
208 inhibitors could be valuable for treating *P. gingivalis* brain colonization and neurodegeneration in AD.

209 Takayama *et al.* [90] investigated the mechanism of AD exacerbation through bacteria-induced microglial  
210 activation. By focally injecting live bacterial cells including *P. gingivalis* into the somatosensory cortex, they showed  
211 that the microglial extension process was strongly induced, along with the production of pro-inflammatory  
212 molecules, by the activated microglia. Interestingly, this process was significantly increased during the light  
213 (waking) phase compared with the dark (sleeping) phase, demonstrating a shared circadian rhythm link in AD  
214 pathology [91]. It was suggested that the bacteria-induced microglial extension was likely initiated through the  
215 secretion of UDP nucleotides in response to the bacteria, which are then taken up by P2Y<sub>6</sub> receptors. Wu *et al.* [92]  
216 also speculated that cathepsin B might play a critical role in the initiation of neuroinflammation and neural  
217 dysfunction following chronic systemic exposure to LPS from *P. gingivalis*.

218 Recently, Carter *et al.* [93] analyzed the *P. gingivalis*/host interactome and showed that misregulated genes  
219 in periodontitis tissue or *P. gingivalis*-infected macrophages also matched those in the AD hippocampus or  
220 atherosclerotic plaques. To this end, the biofilm concept of AD senile plaques has also been proposed, supported by  
221 the hypothesis that curli fibers (and/or other similar bacterial antigens) could aggregate and acquire A $\beta$  conformation  
222 [29,34,44,68,72,76,79,94].

223 In conclusion, *P. gingivalis* is a key oral pathogen and has been suggested to have an important role in the  
224 pathogenesis of neurodegeneration, *via* interaction with iron metabolism, induction by LPS of deleterious  
225 immunological activations (leading to a brain toxic oxidative stress), expression of active specific proteases that

226 enable cleavage of the amyloid- $\beta$  protein precursor and tau resulting in the formation of amyloid- $\beta$  and  
227 neurofibrillary tangles.

228

## 229 2.2 *P. gingivalis* and lung diseases (Figure 2)

230 *P. gingivalis* is able to colonize respiratory airways and reportedly plays a role in aspiration pneumonia  
231 [95], especially in elderly patients and those with a weakened immune system. During aspiration, oral secretions  
232 containing periodontal bacteria may alter the respiratory epithelium and influence respiratory pathogen adhesion  
233 [96]. While oral bacteria such as *Aggregatibacter actinomycetemcomitans* induce the production of pro-  
234 inflammatory cytokines and the recruitment of inflammatory cells, *P. gingivalis* has been found to suppress cytokine  
235 production, which will hinder the host's immunological response against pathogens adhered to the respiratory  
236 epithelium. In addition, endotracheal tubes (ET) form a link between the oral cavity and lungs of intensive care unit  
237 (ICU) patients; oral biofilms have been found in ET, and this forms an association between periodontal disease and  
238 nosocomial pneumonia [97].

239 Chronic obstructive pulmonary disease (COPD), characterized by increased limitation of airflow, can lead  
240 to emphysema, chronic bronchitis, refractory asthma, and some forms of bronchiectasis. A study of the 16S rRNA  
241 subunit using RT-PCR was conducted to analyze bacteria in the dental plaque and lungs of ICU patients with severe  
242 acute exacerbation of COPD. The study results showed that *P. gingivalis* (as with *Pseudomonas aeruginosa*, *P.a.*)  
243 was found at higher rates in the tracheal aspirate samples of ICU patients than in the paired dental plaque samples  
244 [98]. *P. gingivalis* was found directly in the lung [99] but not statistically increased during infection. However, DNA  
245 from *P. gingivalis* was often found in lungs and respiratory infections [100,101], indicating activity remote from the  
246 periodontal bacteria. Another study showed with a multivariate logistic regression analysis that a normal IgG titer  
247 for *P. gingivalis* was correlated with an exacerbation of COPD, whereas the number of exacerbations is significantly  
248 diminished with a higher IgG titer [100]. Therefore, the measurement of *P. gingivalis*-specific IgG antibodies might  
249 be useful to identify patients susceptible to frequent COPD exacerbations.

250 Similar to *P. aeruginosa*, *P. gingivalis* is able to modulate the apoptosis of respiratory epithelial cells [102].  
251 Infection was initiated by adhesion to and invasion of mucosal epithelial cells. *P. gingivalis* and *P. aeruginosa*  
252 induced apoptosis in 12 h and 4 h, respectively. Although the association (co-infection) of *P. gingivalis* and *P.*  
253 *aeruginosa* slowed the process, it also enhanced the invasion ability of each species, and ultimately increased  
254 apoptosis. Indeed, *P. gingivalis* inhibited the *P. aeruginosa*-induced apoptosis in the first hours of the infection by

255 obscuring it from the immune system. The failure of the host defenses to eliminate *P. aeruginosa* allowed the  
256 bacteria to proliferate and destroy tissue during a prolonged infection. Co-infection with *P. gingivalis* and *P.*  
257 *aeruginosa* induced activation of the STAT3 signaling pathway during the first 2 h, following with the up-regulation  
258 of genes for anti-apoptotic survival proteins and *bcl-2*. In addition, pro-apoptotic *bad* was downregulated during the  
259 first 4 h, and caspase-3 activity was inhibited. During this time, *P. gingivalis* and *P. aeruginosa* were able to colonize  
260 more epithelial cells. Subsequently, STAT3 activation decreased while the activation of caspase-3 increased, causing  
261 apoptosis and tissue damage. Furthermore, it has been reported that pyocyanin, a *P. aeruginosa* virulence factor, can  
262 promote heme acquisition [95] and enhance the virulence of *P. gingivalis* by facilitating methemoglobin formation,  
263 leading to a heme-excess condition that upregulates gingipain activity, in particularly Kgp.

264 Aspiration pneumonia is an infection of the lower airways and affects the pulmonary alveoli. It is a life-  
265 threatening condition caused by aspiration of bacteria into the respiratory system during medical procedures, or by  
266 accidental aspiration of liquids (e.g., saliva) or solids (e.g., food). In a study on mice, mixed infections with *P.*  
267 *gingivalis* and *Treponema denticola* caused excessive inflammatory responses compared with mono-infections  
268 [103]. Up to 72 h after inoculation, the number of viable *P. gingivalis* cells in a mixed infection was significantly  
269 higher than after a mono-infection. Mixed infection caused more severe bronchopneumonia by delaying pulmonary  
270 clearance of *P. gingivalis* and caused 40% of death, versus only 10% with mono-infection. In addition, because of  
271 excessive cytokine production leading to shock, lung damage, and tissue dysfunction, 10% of the mice formed an  
272 abscess caused by *P. gingivalis* only. Another mouse study [95] demonstrated the role of gingipains (Kgp and Rgp)  
273 in the development of pneumonia. This study suggested that even in their inactive form, Kgp and Rgp could provoke  
274 strong inflammatory reactions in the host by increasing cytokine production and C-reactive protein (CRP) levels.  
275 Benedyk *et al.* [95] showed, in a mice model, that gingipains were not required for *P. gingivalis* colonization and  
276 survival in the lungs. They were essential for inflammation (neutrophil infiltration) and for manifestation of clinical  
277 symptoms (including hemorrhage, necrosis, and abscess) and infection-related mortality, which were absent from  
278 lungs infected with gingipain-null isogenic strains or WT bacteria preincubated with gingipain-specific inhibitors.  
279 A systemic inflammatory response was observed (high levels of TNF, IL-6, IL-17, and C-reactive protein),  
280 associated with an increase of platelet counts in the blood and activation of platelets in the lungs.

281 In conclusion, *P. gingivalis* can enter into the lower airway following inhalation, aspiration or through  
282 systemic dissemination by bacteremia. In the presence of lung cancer cells, the microenvironment is more conducive  
283 to the survival of the bacterium, which in turn promotes the malignant progression of lung cancer.

284 2.3 *P. gingivalis* in the cardiovascular system (Figure 3)

285 In addition to releasing local inflammatory markers from the periodontium, *P. gingivalis* is able to travel into  
286 the bloodstream. Li *et al.* [104] reported that bacteremia, including anaerobic bacteria, can occur after oral surgery.  
287 A study detected the presence of viable bacteria, including *P. gingivalis*, with the use of invasion assays,  
288 immunofluorescence microscopy, and q-PCR in human atherosclerotic plaque (1 sample only) [105]. Bacterial  
289 viability of *P. gingivalis* was confirmed with invasion assay of non-phagocytic cells. Deshpande *et al.* [106] showed  
290 that *P. gingivalis* can actively adhere to and invade bovine heart and aorta endothelial cells, as well as human  
291 endothelial cells. Examination of human tissue samples (n=50) obtained during carotid endarterectomy revealed that  
292 26% of the surgical specimens were positive for *P. gingivalis* [107], Stelzel *et al.* [108] analyzed aortic tissue  
293 biopsies from 26 patients connected to a heart-lung machine during open-heart surgery. 16S rRNA PCR was used  
294 to detect bacterial cells (bacterial DNA was detected in 88.6% of samples), and specific primers revealed *P.*  
295 *gingivalis* in 15% of examined aortic tissues.

296 Several mechanisms link *P. gingivalis* to cardiovascular disease: platelet aggregation due to arginine  
297 gingipains, exaggerated host immune response due to *P. gingivalis* LPS, and bacterial inflammatory products of  
298 periodontitis [109]. *P. gingivalis* induces platelet aggregation, which leads to thrombus formation [110]. Loubakos  
299 *et al.* [111] studied the arginine gingipains (RgpA and RgpB), which were released in large quantities from *P.*  
300 *gingivalis*. After entering the circulation, the arginine gingipains increased the intracellular calcium level in platelets  
301 by activating both PAR-1 and PAR-4 receptors. Arginine gingipains also activated factor X, prothrombin, and  
302 protein C, promoting a thrombotic tendency through the release of thrombin, subsequent platelet aggregation,  
303 conversion of fibrinogen to fibrin, and intravascular clot formation. One study on hyperlipidemic mice determined  
304 that oral infection with wild-type (WT) *P. gingivalis* (detected by 16S rRNA PCR) significantly increased the area  
305 of the aortic sinus that was covered with atherosclerotic plaques compared with *P. gingivalis* mutants inactivated  
306 with gingipain or FimA [112]. WT *P. gingivalis* activated innate immune cells through the NLRP3 inflammasome  
307 (NOD-like receptor pyrin domain 3, including pro-IL-1 $\beta$ , pro-IL-18 and pro-caspase 1), which led to sustained  
308 inflammation.

309 LPS induces major vascular responses [104], including infiltration of inflammatory cells, vascular smooth  
310 muscle proliferation, vascular fatty degeneration, and intravascular coagulation. LPS upregulate the expression of  
311 endothelial cell adhesion molecules and the secretion of IL-1 $\beta$ , tumor necrosis factor alpha (TNF- $\alpha$ ), low-density  
312 lipoprotein (LDL) and thromboxane, which results in the recruitment of macrophages, platelet aggregation and

313 adhesion, foam cell formation, and cholesterol deposition. Then IL-1 $\beta$  promoted enhanced CD36/SR-B2 and TLR2  
314 (LPS receptor). In parallel, *P. gingivalis* LPS inhibited pyroptosis, which enabled macrophage survival and promoted  
315 the development of atherosclerotic plaques.

316 The detection of *P. gingivalis* has been attested in many cardiovascular diseases, such as atherosclerosis,  
317 myocardial infarction, stroke, aneurysm, pericarditis, and tamponade [113,114].

318 In atherosclerosis, the surface of atheroma enhances platelet aggregation and thrombus formation, which  
319 can occlude the artery or be released to cause thrombosis, coronary heart disease, and stroke. *P. gingivalis* infection  
320 itself does not cause atherosclerosis [115], but can accelerate it. In turn, that acceleration leads to inflammation  
321 and deteriorating lipid metabolism, particularly in the context of underlying hyperlipidemia or susceptibility to  
322 hyperlipidemia [116]. Some studies have shown that *P. gingivalis* infection accelerates atherosclerosis in  
323 hyperlipidemic animals [112,117-120] and humans [107,121]. Viable *P. gingivalis* were detected in the aortic wall  
324 of mice (on the luminal side and the adventitia) by fluorescence in situ hybridization [119]. That study noted the  
325 presence of macrophages and select classes of T cells in early lesions. Then the study also determined that levels  
326 of total cholesterol, very low-density lipoprotein (VLDL) particles, chylomicrons (CM), LDL, and high-density  
327 lipoprotein (HDL) particles were slightly increased in *P. gingivalis*-infected mice, but remained comparable to  
328 corresponding levels in control mice. In humans, a study came to the same conclusion regarding non-obese patients  
329 with type 2 diabetes [121]: total serum triglycerides and LDL cholesterol levels were higher in patients with a high  
330 *P. gingivalis* IgG titer than in those with a normal IgG titer, but there were no statistically significant differences  
331 between the 2 groups. Only the oxidized LDL (plaque area) significantly increased, and HDL cholesterol decreased  
332 [115]. This shift in the lipid profile was concomitant with a significant increase in atherosclerotic lesions. Indeed,  
333 stimulation with *P. gingivalis* LPS induced a change in cholesterol transport by targeting the expression of LDL  
334 receptor-related genes, resulting in the disturbance of regulatory mechanisms that control cholesterol level in  
335 macrophages. This can occur without any predisposition to high cholesterol. Furthermore, a study on pigs  
336 (histomorphometry and immunohistochemistry), showed a significant increase of atherosclerotic lesions (coronary  
337 and aortic) in infected pigs with normal cholesterol, and an increase in intimal plaque and lesions in the  
338 hypercholesterolemic group that neared statistical significance when compared with controls [118]. Diet is an  
339 aggravating factor for atherosclerosis, but regardless of diet, a *P. gingivalis* infection can accentuate atherosclerosis  
340 lesions. In a murine study [117], *P. gingivalis* was detected in the proximal aorta by 16S rRNA PCR, while  
341 inflammatory markers were detected using ELISA. 24 weeks after inoculation, the proximal aortic lesion size

342 (quantified by histomorphometry) was 9-fold greater in chow-fed infected mice than in controls, and was 2-fold  
343 greater in *P. gingivalis*-inoculated mice versus non-inoculated mice that were fed a high-fat diet.

344 Myocardial infarction is the damage or necrosis of an area of heart muscle due to reduced blood supply  
345 to that area. An article that studied the bacterial signatures in thrombus aspirates from patients with myocardial  
346 infarction [122] measured endodontic bacteria in 78.2% of thrombi, periodontal pathogens in 34.7%, and detected  
347 *P. gingivalis* in 5%. Matrix metalloproteinase (MMP)-9 (produced by leukocytes) levels directly correlate with  
348 dysfunction and remodeling of the left ventricle (LV) post-myocardial infarction, and modulates inflammation.  
349 Exposure to *P. gingivalis* lipopolysaccharide was shown to increase LV MMP-9 levels in mice and leads to cardiac  
350 inflammation [123]. After intravenously inoculation of *P. gingivalis*, myocarditis and/or myocardial infarction were  
351 observed, and Akamatsu *et al.* [124] showed that IL-17A plays an important role in the pathogenesis of these  
352 diseases. High mobility group box 1 (HMGB1), a nuclear protein released from necrotic cells, inducing  
353 inflammatory responses was detected in degenerated cardiomyocytes, extracellular fields, immune cells, and  
354 vascular endothelial cells [125].

355 Stroke or acute cerebral infarction is a cerebrovascular disease that affects blood vessels supplying blood to the  
356 brain. *P. gingivalis* binds to the vascular endothelium via major and minor fimbriae and the infected vascular  
357 endothelium responds by the production of cytokines, chemokines, and surface molecules. *P. gingivalis* enters  
358 immune cells such as monocytes/macrophages or dendritic cells in the oral mucosal lesion, enter the circulation and  
359 diapedesis into the vascular intima. Immune cells activated by *P. gingivalis* drive modification of low-density  
360 lipoproteins and ischemic event comes from occlusive thrombus [126]. In a human study of a Chinese population  
361 [127], levels of *P. gingivalis* IgG antibodies were significantly higher in individuals with acute cerebral infarction  
362 than in healthy controls. *P. gingivalis* IgG titers also correlated significantly with total cholesterol, LDL, and  
363 apolipoprotein-B.

364 An aneurysm is a localized, blood-filled evagination in the wall of a blood vessel. A study compared the  
365 number of human aortic aneurysms between *P. gingivalis*-infected and uninfected groups [128]. It concluded a  
366 higher number of aneurysms occurred in the distal aorta in the *P. gingivalis*-infected group than in the control group.  
367 Cellular accumulation of adipocytes in aneurysms was identified less frequently in the infected group; the expression  
368 of a phenotypic marker for proliferative smooth muscle cells that tends to weaken vascular walls (embryonic myosin  
369 heavy chain isoform) was higher in the *P. gingivalis*-infected group than the non-infected group. In a similar study  
370 [129], *P. gingivalis* was detected in 23.1% of aortic aneurysms. *P. gingivalis* infection decreased HDL levels and  
371 provoked atherosclerosis, thrombosis, and then aneurysm. HDLs displayed multiple functions, including anti-

372 inflammatory and LPS scavenging properties. With this reason, Delbosc *et al.* [130] injected HDL into rats, and the  
373 resulting reduction of neutrophil activation in *P. gingivalis*-injected rats was associated with decreased cytokine  
374 levels in conditioned media and plasma.

375 Pericarditis is an inflammation of the pericardium, which is composed of two thin layers of tissue that  
376 surround the heart, holding it in place and helping it work. A small amount of fluid keeps the layers separate so there  
377 is no friction between them. A tamponade is an acute heart compression, usually due to a pericardial effusion. A  
378 recent study demonstrated the presence of DNA from oral bacteria in the pericardial fluid [131]. In a case report  
379 [132] *P. gingivalis* was detected in the pericardial fluid of a 45-year-old man who developed tamponade 10 weeks  
380 after angioplasty and insertion of an endoprosthesis for myocardial infarction (using 16S rRNA investigation, and  
381 universal amplification with ribotyping).

382 In conclusion, oral bacteremia lead to systemic dissemination of *P. gingivalis* that seems to be markedly  
383 involved in the atherosclerotic process, contributing to LDL oxidation, and this being facilitated by fimbriae  
384 adhesion to aneurysmal tissue, biofilm formation in vessels and activation of inflammasomes.

385

#### 386 2.4 *P. gingivalis* and bone diseases

387 Epidemiological studies suggest that the prevalence of rheumatoid arthritis (RA) (autoimmune disease)  
388 and periodontitis (infectious etiology) are superposable [133]. *P. gingivalis* has a strong association with  
389 progressive periodontal disease and RA [134,135] linked to an induction of local immune system disorders  
390 [136,137]. A significant association between the presence of *P. gingivalis*, halitosis and RA was demonstrated and  
391 linked to biofilm formation on the tongue and increased inflammation leading to more active disease [138]. The  
392 association between rheumatoid arthritis (RA) and periodontal disease (PD) has been the subject of numerous studies  
393 justified by common inflammatory processes and the presence of anti-citrullinated protein autoantibodies (ACPA).  
394 RA is an autoimmune disease characterized by chronic inflammation, causing inflammation and destruction of the  
395 synovial joints. PD is a chronic inflammatory disease associated with dysbiosis of oral microbiota affecting the  
396 support tissues around the teeth, resulting in the destruction of connective tissue and loss of teeth. *P. gingivalis* has  
397 been implicated in the generation of ACPA and studies show the clinical link between PR and DR and the possible  
398 similar mechanisms linking the development and progression of the two diseases [139]. *P. gingivalis* produces the  
399 enzyme PAD, cysteine proteinases, or other gingipains [140-142]. which induce citrullination of different  
400 autoantigens (levels of anti-citrullinated protein antibodies are considerably higher in RA patients with than in those

401 without periodontal disease) [143]. These results were recently confirmed on a mice model [144]. Dysregulation  
402 would induce tissue destruction and bone resorption. The presence of autoimmune anticitrullinated protein  
403 antibodies (ACPA) during preclinical stages of disease and accumulation of hypercitrullinated proteins in arthritic  
404 joints has been demonstrated. Protein citrullination causes deregulation of the host's inflammatory signaling network  
405 by altering the spatial arrangement of the original 3D-structure and function of the protein [145]. *P. gingivalis* is  
406 able to citrullinate some proteins, as peptidyl arginine deiminase (PAD), and anti-PAD antibodies to *P. gingivalis*  
407 have been identified in the serum of RA patients and patients with periodontitis. The levels of antibodies against *P.*  
408 *gingivalis* in the sera and synovial fluid of RA and non-RA patients were comparable to those found in respective  
409 controls [146], and PAD was demonstrated not to be a major virulence mechanism during early stages of  
410 inflammatory arthritis, suggesting that other mechanisms might be involved [147]. However, *P. gingivalis* DNA  
411 was detected in the synovial tissue of RA patients, suggesting the possible intracellular localization of the bacteria  
412 [148-152]. A significant correlation has been observed between osteoporosis and missing teeth, with dysbiosis and  
413 the detection of *P. gingivalis* in 98.7% [153]. The *P. gingivalis* capsule also seems to play a role: using a murine  
414 model of periodontitis, Monasterio *et al.* [154] have shown that bone resorption was less important in mutated mice,  
415 due to decreased Th1 / Th17 immune response and less osteoclast activity.

416           When the gingival immune response is impaired, matrix metalloproteinases are released from neutrophils  
417 and T cells, mediating alveolar bone loss [155]. Monocytes in inflamed tissues differentiate into osteoclasts, and  
418 also accelerate bone resorption through the production of pro-inflammatory mediators [126,156]. The pro-  
419 inflammatory cytokine IL-17, and IL-17-producing CD4+ T cells (also called helper T, or Th17, cells) have been  
420 detected in periodontal disease lesions in humans, especially those associated with disease severity [157,158]. TLR2  
421 and TNF mediate destructive effects in oral infection and oral bone loss [159]. Chemokine antagonist treatments  
422 have been employed successfully to improve periodontitis [160,161]. The prevalence of periodontal disease is higher  
423 in RA patients than in the general population, linked to an increased prevalence of the shared epitope HLA-DRB1-  
424 04 and various immunological disorders. TNF- $\alpha$  antagonists or biotherapies slow alveolar resorption, but may  
425 maintain infection in periodontal pockets [162]. *P. gingivalis* carries heat shock proteins (HSPs) that may also trigger  
426 autoimmune responses in subjects with RA [133].

427           The complement is also involved in the dysbiosis of the periodontal microbiota and inflammation leading  
428 to the destruction of periodontal bone [163]. This gingival inflammation resulted in alveolar bone resorption and  
429 loss of periodontal ligament [164], leading to continued loss of attachment. This has been confirmed in animal  
430 models, with alveolar bone loss induced by inoculation or injection of human oral bacteria (e.g., *P. gingivalis*) in



431 different animal models [165]. Oral infection in mice with *P. gingivalis* was demonstrated to induce alveolar bone  
432 loss [126,166].

433 In conclusion, *P. gingivalis* can induce citrullination, and production of anticitrullinated antibodies, often  
434 detected in rheumatoid arthritis and other auto-immune diseases. *P. gingivalis* can also induce osteoclastogenesis,  
435 and Th17 proinflammatory response leading to bone damage and systemic inflammation.

436

#### 437 2.5 *P. gingivalis* in the digestive system

438 Because of saliva and the continuity between the mouth and the gut, a large quantity of oral bacteria is  
439 constantly ingested [167]. *P. gingivalis* may invade or alter the gut microbiota, thereby leading to increased  
440 permeability of the gut epithelium and endotoxemia, which in turn can cause systemic inflammation and disease.

441 A study showed the presence of *P. gingivalis* in esophagus squamous cell carcinoma (ESCC) [168]. This  
442 study proceeded to qRT-PCR to detect *P. gingivalis* 16S rRNA by using antibodies targeting whole bacteria and the  
443 gingipain Kgp. *P. gingivalis* was detected immunohistochemically in 61% of cancerous tissues and 12% of adjacent  
444 tissues; it was not detected in normal esophageal mucosa. In metastatic cancers, the percentage of infection by *P.*  
445 *gingivalis* rose to 84.2%.

446 FimA protein from *P. gingivalis* was found in 50% of saliva specimens from patients with liver cirrhosis or  
447 a previous round of treatment for hepatocellular carcinoma, compared with 21.43% in patients with hepatitis C  
448 alone. This *in vivo* human study [169] supposed that periodontal disease might be associated with the progression  
449 of viral liver disease, but no studies revealed *P. gingivalis* in the liver. Pyogenic liver abscess (PLA) formation has  
450 three major infectious transmission paths: the biliary tract, portal vein, and hepatic artery; however, 50% are  
451 cryptogenic (unknown origin). One case report [170] demonstrated that periodontal infection was a potential source  
452 of infection in PLA formation. Cryptogenic PLA presented in the autopsy of a 59-year-old woman who suddenly  
453 developed cardiopulmonary arrest and died. She had also had periodontitis for a long time. Immunohistochemical  
454 staining of a liver puncture and other samples detected isolated *P. gingivalis* and other periodontal pathogens in this  
455 patient's PLA, heart, and kidney, as well as in the thrombus.

456 Hepatic steatosis in patients with nonalcoholic fatty liver disease (NAFLD) is characterized by intracellular  
457 accumulation of lipid droplets (triglycerides) in hepatocytes. It is widely regarded as being the hepatic manifestation  
458 of conditions related to metabolic syndromes [171], including type 2 diabetes, insulin resistance, obesity,

459 hyperlipidemia, hypertriglyceridemia, and hypertension. Studies on mice [172] revealed that infection with *P.*  
460 *gingivalis* promoted the pathological progression of NAFLD. Internalized *P. gingivalis* was localized in  
461 autophagosomes and lysosomes in HepG2 cells, rather than in liquid droplets. However, lipid droplets increased the  
462 existence of *P. gingivalis* in HepG2 cells at an early phase of infection and affected the formation of autolysosomes  
463 to eliminate *P. gingivalis*. Thus, lipid droplets were likely to affect the persistence of *P. gingivalis* in HepG2 cells  
464 by altering the autophagy-lysosomal system.

465 Nonalcoholic steatohepatitis (NASH) is a subtype of NAFLD in patients that have hepatic lesions and  
466 inflammation in addition to an excess of fats (NASH-hepatitis). A study on mice conducted by Furusho *et al.* [173],  
467 using histological and immunohistochemical examinations, measured LPS levels in the liver. Cytokines were studied  
468 by ELISA, which determined that *P. gingivalis* led to the upregulation of its LPS receptor (TLR2), formation of  
469 areas of fibrosis, proliferation of hepatic cells, and collagen formation.

470 The effect of bacteria and inflammation on the gallbladder is capital. Indeed, as cirrhosis progresses,  
471 modifications of the microbiome can lead to inflammation. On the other hand, the LPS of *P. gingivalis* has been  
472 shown to induce TNF- $\alpha$  through TLR2 and TLR4. Inflammation suppressed synthesis of the bile acid deoxycholic  
473 acid [174] and modified the intestinal and colonic pH, favoring a positive feedback mechanism and resulting in the  
474 overgrowth of pro-inflammatory members of the microbiome, including *P. gingivalis*.

475 Pancreatic cancer is the fourth leading cause of cancer deaths worldwide. Anti-*P. gingivalis* antibody titers  
476 are reportedly higher in patients with pancreatic cancer than in healthy controls [175]. This finding resulted in the  
477 hypothesis that PAD enzymes found in oral bacteria, and especially in *P. gingivalis*, might cause the p53 point  
478 mutations observed in patients with pancreatic cancer. This hypothesis has not yet been tested, and there is a need  
479 to detect *P. gingivalis* DNA or anti *P. gingivalis* antibody in patients with a p53 arginine mutation. However, a  
480 recent murine study [176] showed that *P. gingivalis* did not need to be present in the tissue where the tumor  
481 developed or cause inflammation in the tumor tissue in order to promote carcinogenesis. In mouse models [176], the  
482 LPS of *P. gingivalis* was able to accelerate pancreatic carcinogenesis through TLR4 activation (in addition to TLR2).  
483 TLRs inhibited apoptosis and promoted tumor growth and angiogenesis. LPS increased the invasive ability of  
484 pancreatic cancer cells.

485 Animal studies have suggested that periodontitis accelerates the onset of hyperinsulinemia and insulin  
486 resistance [177]. The LPS of *P. gingivalis* stimulated serpine 1 expression in the pancreatic beta cell line MIN6 and  
487 induced insulin secretion. To prove that, an *in vitro* study (using cell culture, ELISA, and real-time PCR) co-

488 incubated *P. gingivalis* with MIN6 cells and measured the effect on insulin secretion. In addition, the involvement  
489 of serpine 1 in insulin secretion was determined by downregulating serpine 1 expression. It was demonstrated that  
490 *P. gingivalis* was able to stimulate insulin by 3-fold in a normoglycemic context and 1.5-fold in a hyperglycemic  
491 context. *P. gingivalis* significantly upregulated expression of the serpine 1 gene, which causes an increase in insulin  
492 secretion by MIN6 cells. Cells with downregulated serpine1 expression were resistant to the effects of *P. gingivalis*  
493 on insulin stimulation under normoglycemic conditions.

494 Inflammatory bowel disease (IBD) and diseases of the small intestine were correlated with periodontal risk  
495 and bad oral health [178]. In a mouse model, oral administration of *P. gingivalis* induced changes in the composition  
496 of the gut microbiota associated with epithelial alteration and cell barrier function (increased phylum Bacteroidetes,  
497 decreased phylum Firmicutes) [179]. *P. gingivalis* administration also highlighted insulin resistance and changes of  
498 gene expression in adipose tissue and liver, but this study did not detect *P. gingivalis* in the gut. *P. gingivalis*  
499 downregulated tight junction protein 1, occluding expression in the small intestine, and upregulated IL-6 expression.  
500 In the large intestine, *P. gingivalis* increased TNF- $\alpha$  expression.

501 In acute appendicitis in children [180], sequencing of one sample among 22 (concerning a perforated  
502 appendicitis) demonstrated that *P. gingivalis* was the most abundant species (1 sample contained 63%, other  
503 contained <1%, and non-appendicitis samples contained 0%). In another *in vivo* study, *Porphyromonas* spp. was  
504 cultured in 16.7% of surveyed patients with gangrenous appendicitis [181]. In another study of gangrenous or  
505 perforated appendicitis, a Wadsworth experience [182] with black-pigmented anaerobes isolated 5 strains of *P.*  
506 *gingivalis* among 76 patients.

507 In a human *in vivo* study [183], *Bacteroides-Prevotella-Porphyromonas* was significantly more abundant in  
508 patients with ulcerative colitis (UC) or Crohn's Disease (CD) than in controls. As for *P. gingivalis*, Bibiloni *et al.*  
509 [184] reported an increase of *Porphyromonas* species in biopsies from UC patients, without specifying the  
510 mechanisms.

511 Studies have shown that the CRC risk is linked to decreased bacterial diversity in feces [185]. One study of  
512 human fecal samples (16S rRNA PCR and pyrosequencing) showed a decrease of *Clostridia* (Gram positive, fiber-  
513 fermenting) and increased *Fusobacterium* and *Porphyromonas* (Gram negative, pro-inflammatory). These were  
514 already known to synergistically promote oral cancer [186]. Here, 16S rRNA gene sequencing results [186]  
515 suggested that *F. nucleatum* and *Porphyromonas* spp. form a bacterial biofilm on the tumor. To confirm this  
516 hypothesis, another study [187] showed that detection of *F. nucleatum* and *P. gingivalis* metabolites was linked to

517 the number of CRC with these high-risk microbes (29% and 34%, respectively). Cultivation of mouse colon  
518 carcinoma cells showed that *P. gingivalis* GroEL protein (a chaperone protein), as a virulence factor, accelerated  
519 tumor growth [188] (volume and weight) by enhancing endothelial progenitor cell function and neovascularization.

520 In conclusion, *P. gingivalis* can induce a persistent elevation in the cytokine response linked to type 2  
521 diabetes, that leads to dysregulation of lipid metabolism and effects on pancreatic beta cells. Particularly, TNF-  
522 alpha, a potent inhibitor of the tyrosine kinase activity of the insulin receptor, has been implicated as an etiologic  
523 factor for insulin resistance. Inflammation can also contribute to formation of areas of fibrosis, proliferation of  
524 hepatic cells, collagen formation and cancerization.

525

## 526 2.6 *P. gingivalis* and genital or perinatal infections

527 The relationship between adverse pregnancy outcomes and maternal antibody response to *P. gingivalis* was  
528 studied by Sasahara *et al.* [189], who demonstrated that a lack of humoral immunity against *P. gingivalis* during  
529 early pregnancy was associated with intrauterine growth retardation and some preterm birth. In Lebanon, *P.*  
530 *gingivalis* was detected in the oral plaque of pregnant subjects (65%) with clinical periodontitis compared with a  
531 healthy periodontium. However, none of the 20 study participants experienced preterm labor [190], and the authors  
532 concluded that these preliminary results did not indicate a relationship among *P. gingivalis*, periodontitis, CRP  
533 levels, and preterm labor. Other authors [191,192] confirmed that there was no significant correlation between the  
534 presence of *P. gingivalis* in the placenta and perinatal complications. Ibrahim *et al.* [193] studied the association  
535 between *P. gingivalis* infection and recurrent miscarriage. The prevalence of *P. gingivalis* DNA in chorionic villous  
536 tissue samples and in cervicovaginal secretions was significantly higher in the case of recurrent early miscarriage  
537 (16% and 18%, respectively) than in women with no such history.

538 In conclusion, *P. gingivalis* can directly injure and alter the morphology of the foetal and maternal tissues, by  
539 increasing the production of cytokines and free radicals in the uterine compartment, that could increase the risk of  
540 myometrial contraction and onset of preterm labor.

541

## 542 2.7 *P. gingivalis* and cooperation with other oral bacteria

543 If *P. gingivalis* plays a crucial role in oral pathologies, studies of microbiome have shown that inter-  
544 bacterial cooperation is also possible, most often leading to an increase in its abundance [194] or/ and pathogenic  
545 power [195]. In the colon these bacteria can alter the composition of the residual microbiota, in the context of  
546 complex biofilms, resulting in intestinal dysbiosis by production of virulence factors creating bacterial instability  
547 and outgrowth of pathogens [196]. Cooperative biofilm formation and motility by *Streptococcus gordonii*,  
548 *Fusobacterium nucleatum*, *Treponema denticola* and *Porphyromonas gingivalis* were particularly documented *in*  
549 *vitro*, using growth models and scanning electron microscopy [197,198], and confirmed by metagenomics analysis  
550 [199]. Within an oral biofilm *P. gingivalis* is capable of increasing the expression of its fimbriae and facilitating the  
551 invasion of *F. nucleatum* into dendritic cells [200]. Communities of *P. gingivalis* with the accessory pathogen  
552 *S. gordonii*, which are synergistically pathogenic, show an increase in expression of genes encoding T9SS  
553 components [201]. The 4-aminobenzoate/para-amino benzoic acid (pABA) synthesized by *S. gordonii* is required  
554 for maximal accumulation of *P. gingivalis* in dual-species communities. Metabolomic and proteomic data showed  
555 *P. gingivalis* leads to decreased stress, virulence *in vivo*, extracellular polysaccharide production by *P. gingivalis*,  
556 but promote increase expression of fimbrial adhesins, colonization and survival of *P. gingivalis* in a murine oral  
557 infection model [202]. The chymotrypsin-like proteinase (CTLP) or dentilisin, a virulence factor of *T. denticola*,  
558 was shown to mediate its adherence to other potential periodontal pathogens, *P. gingivalis*, *F. nucleatum*, *Prevotella*  
559 *intermedia* and *Parvimonas micra* [203].

560

### 561 3 CONCLUSION

562 Many virulence factors produced by *P. gingivalis*, such as lipopolysaccharide, capsule, fimbriae,  
563 gingipains, adhesins, outer membrane vesicles, interleukin-8 (IL-8) production induced by "Hemoglobin receptor  
564 (HbR), have been demonstrated in oral pathologies, inducing chronic inflammation and alveolar bone resorption. *P.*  
565 *gingivalis* is also able to indirectly act on DNA by inhibiting enzymes involved in its synthesis or activity. Concurrent  
566 studies support the role of *P. gingivalis* in many systemic diseases. Recent work on microbiota confirmed the first  
567 studies by PCR-sequencing and cultures. We hope that, in the future, new technologies (e.g., those studying the  
568 microbiome and bacterial metabolites) will be able to confirm these many assumptions, which have been put forward  
569 about the major role of *P. gingivalis*, as well as the intra-bacterial cooperation in the genesis of systemic diseases.

570

### 571 ACKNOWLEDGMENTS

572 We thank Adina Pascu for helping us to edit the manuscript.

## 573 CONFLICT OF INTEREST STATEMENT

574 The authors declare they have no conflicts of interest.

575

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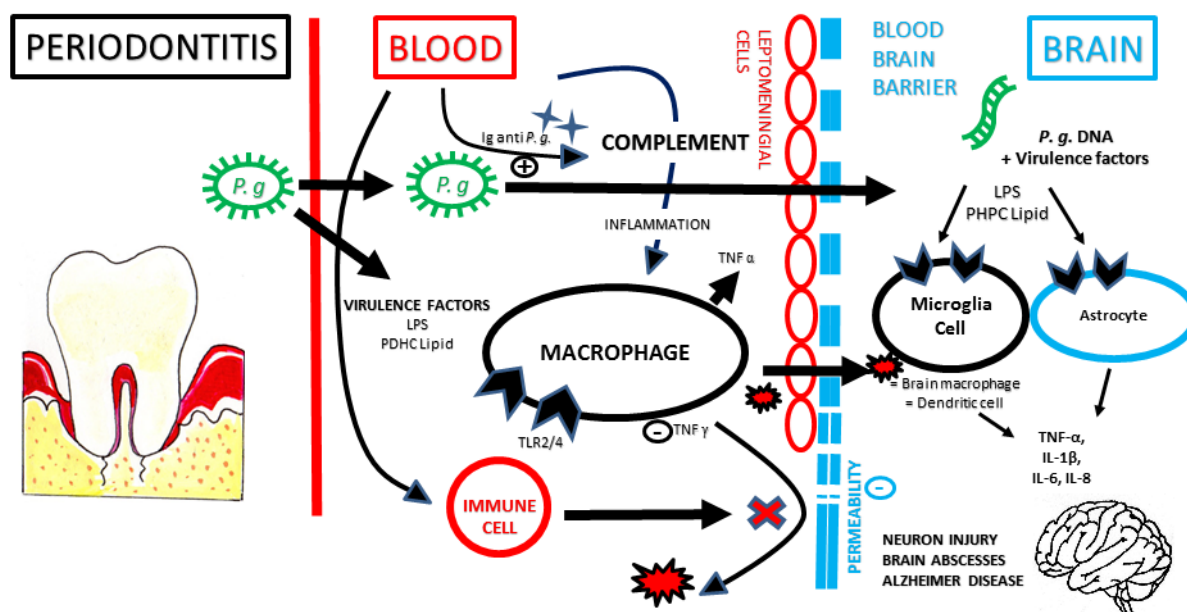
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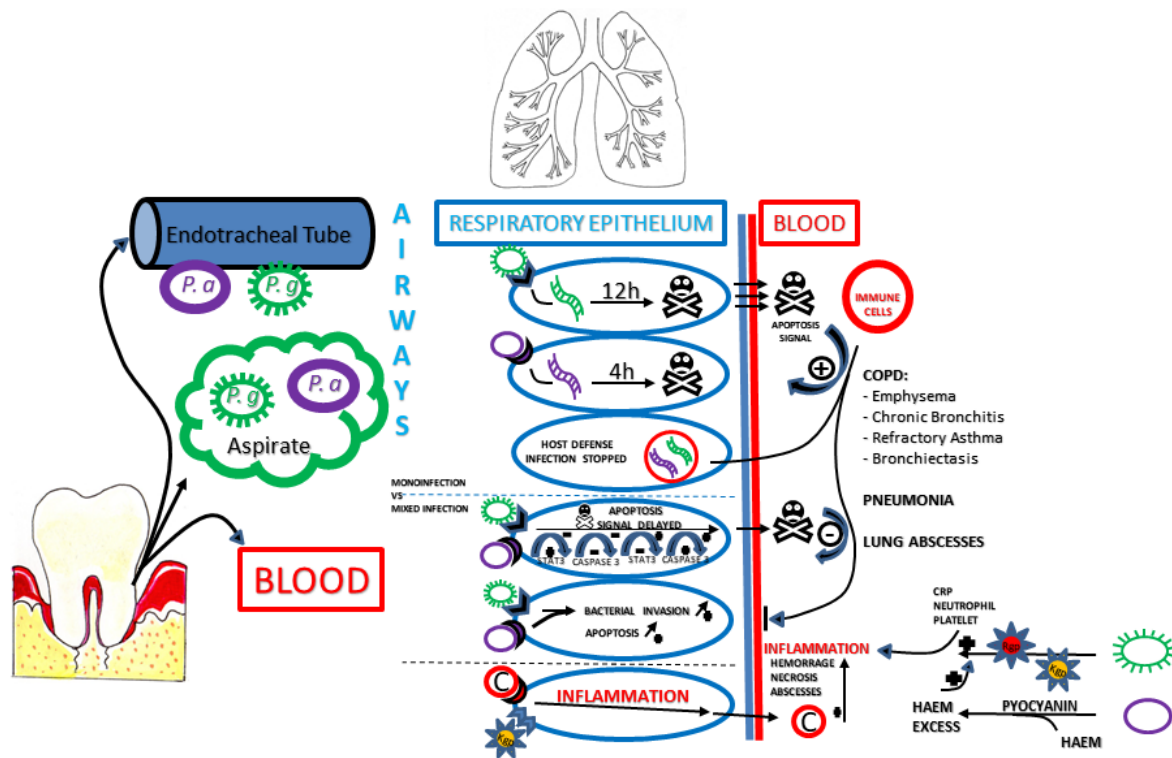


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1182 **Figure 1.** *Porphyromonas gingivalis* (*P. gingivalis*) and brain diseases: molecular mechanisms involved.

1183 *P. gingivalis* can easily access the systemic circulation in periodontitis patients. A relationship between *P. gingivalis*-  
 1184 specific antibodies in serum and neurologic disorders has been pointed out by several studies. Once it reaches the  
 1185 bloodstream, *P. gingivalis* can induce an impaired inflammatory state via its virulence factors and secreted  
 1186 molecules from macrophages and immune cells. The expression of TLR2, TLR4, TNF- $\alpha$  and inducible nitric oxide  
 1187 synthase (iNOS) is thought to be induced by the LPS of *P. gingivalis* in M1 macrophages of chronic periodontitis  
 1188 patients. Pro-inflammatory signals are communicated to microglia through leptomeningeal cells. The presence of  
 1189 LPS from *P. gingivalis* can be detected in brain tissue (post-mortem brain dementia) and on the surface membrane  
 1190 of astrocytes, and could therefore raise neuro-inflammation, which is strongly implicated in Alzheimer's disease. *P.*  
 1191 *gingivalis* may also affect the blood-brain barrier in Alzheimer's patients, which may explain the detection of  
 1192 bacterial DNA and LPS in brain tissues. In turn, these virulence factors may enhance neuro-inflammation and  
 1193 neuronal damage, with increased expression of inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8.

1194 IL-6: interleukin 6; LPS: lipopolysaccharide; PHPC lipid: 1-palmitoyl-2-hexadecyl-sn-glycero-3-phosphocholine  
 1195 lipid; TLR2/4: Toll-like receptors 2 and 4; TNF- $\alpha$ , TNF- $\gamma$ : tumor necrosis factors  $\alpha$  and  $\gamma$ .



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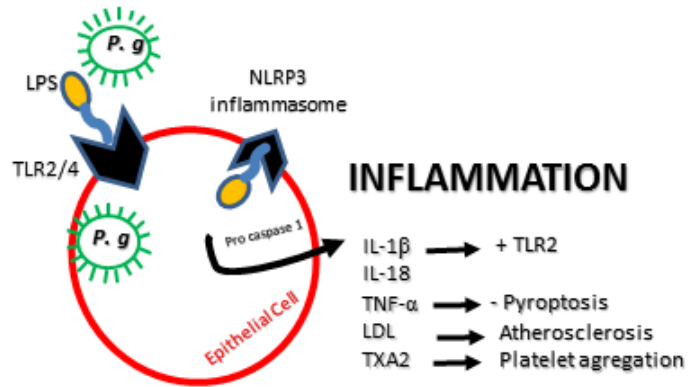
1197 **Figure 2.** *P. gingivalis* and lung diseases: molecular mechanisms involved.

1198 During aspiration, oral secretions containing periopathogenic bacteria may spread in the respiratory airways.  
 1199 Bacteria involved in respiratory diseases (e. g., cystic fibrosis), such as *P. gingivalis* (*P. g*) or *Pseudomonas*  
 1200 *aeruginosa* (*P. a*), may also colonize the respiratory tree through endotracheal tubes. As in gingival tissues, *P.*  
 1201 *gingivalis* can adhere to and invade respiratory epithelial cells, escaping the host defense and modulating apoptosis.  
 1202 *P. gingivalis* and *P. aeruginosa* induced apoptosis in 12 h and 4 h, respectively. Furthermore, a mixed infection with  
 1203 a common opportunistic pathogen of the respiratory system (*P. aeruginosa*) may lead to bacterial synergy to promote  
 1204 inflammation (increase of CRP; polynuclear recruitment; putative role of gingipains Rgp and Kgp for *P.g*; or  
 1205 pyocyanin for *P.a*) and ultimately facilitate the pathogenesis of respiratory diseases.

1206 COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein, C: complement activation.

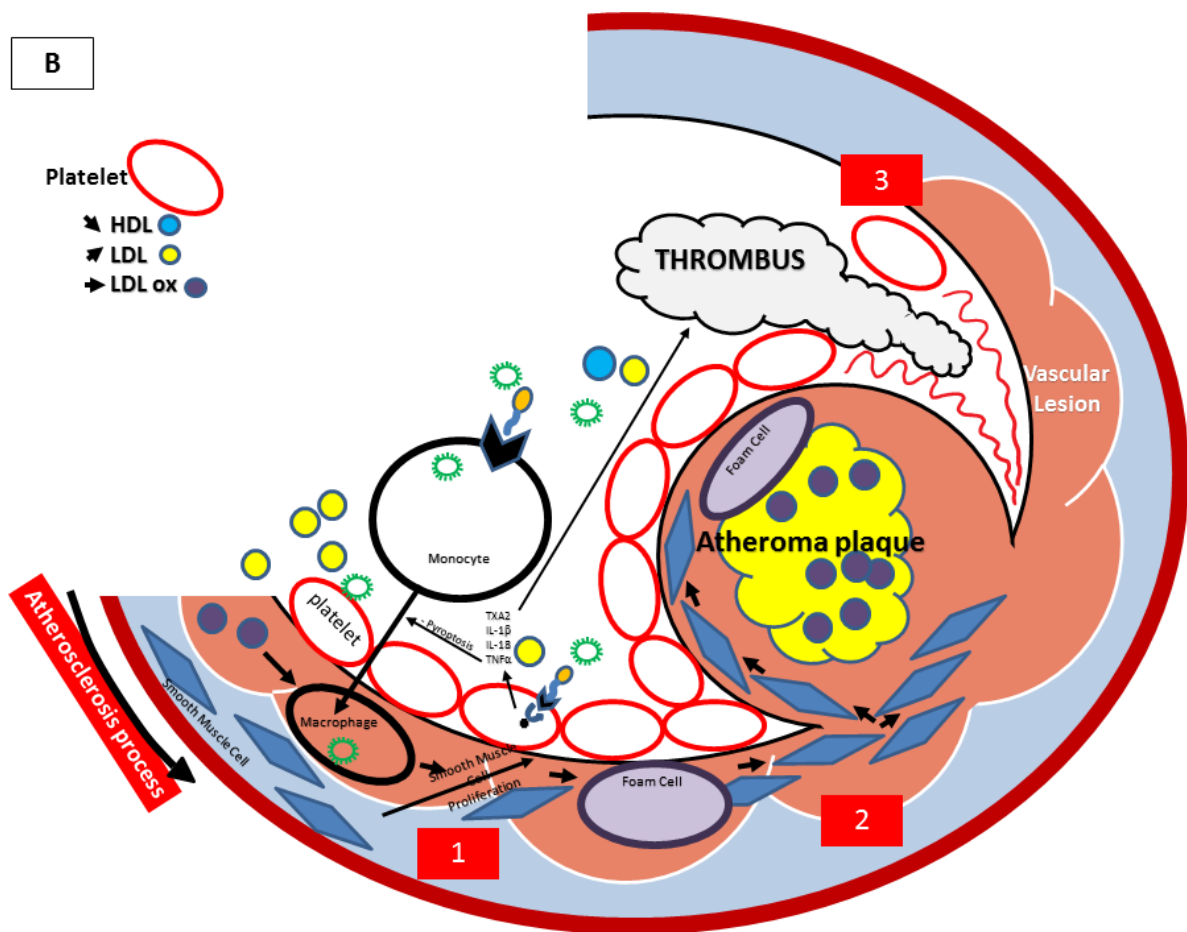
A

**1** Inflammatory infiltration  
Smooth muscle proliferation



1207

B



1208

34

1209 **Figure 3.** *P. gingivalis* and atherosclerosis: molecular mechanisms involved.

1210 Following bacteremia, *P. gingivalis* can travel from the oral cavity through the bloodstream to the heart and aorta,  
1211 adhering to endothelial cells.

1212 A1 and B 1: *P. gingivalis* adheres to endothelial cells, inducing inflammation and cytokine liberation. LPS also  
1213 induces an increased immune response, with liberation of cytokines.

1214 A2: Gingipains from *P. gingivalis* can activate platelets and induce their aggregation, as well as foam cell formation  
1215 and cholesterol deposition. Atherosclerosis is an inflammatory disease stimulated by LPS from *P. gingivalis*, which  
1216 can inhibit pyroptosis, enabling macrophage survival and promoting the development of atherosclerosis plaques.

1217 A3: A shift in the lipid profile (oxidized LDL in the plaque area significantly increased, and HDL cholesterol  
1218 decreased) was concomitant with a significant increase in atherosclerotic lesions. Induced inflammation and vascular  
1219 lesions promote thrombus formation.

1220

1221 HDL: high-density lipoprotein; IL-1 $\beta$ , IL-18: interleukins 1 $\beta$  and 18; LDL: low-density lipoprotein; NLRP3:  
1222 cryopyrin; TLR2/4: Toll-like receptors 2 and 4; TNF- $\alpha$ : tumor necrosis factor  $\alpha$ ; TXA2: thromboxane A2.

1223