ACADEMIE d'AIX-MARSEILLE

Proposition d'un modèle d'évaluation clinique de sévérité des péri-implantites

THESE

Présentée et publiquement soutenue devant la

Faculté d'Odontologie de Marseille
(Doyen : Monsieur le Professeur Jacques DEJOU)

Aix Marseille Université
(Président : Monsieur le Professeur Yvon BERLAND)

Le 13 octobre 2017

par

MAZEL Anthony
né le 03 janvier 1991
à La Seyne-sur-Mer

Pour obtenir le Diplôme d'Etat de Docteur en Chirurgie Dentaire

EXAMINATEURS DE LA THESE :

Président	: Monsieur le Professeur	M. RUQUET
Assesseurs	: Monsieur le Professeur	M. DRANCOURT
	Monsieur le Docteur	G. ABOUDHARAM
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Habilitation à diriger des recherches

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I. INTRODUCTION

Lors de l'insertion d'un implant, après obtention de l'ostéo-intégration, des complications inflammatoires d'origine infectieuses peuvent apparaître et affecter les tissus péri-implantaires. Le premier stade de ces complications est la mucosite ou inflammation de la muqueuse sans perte osseuse. La mucosite est réversible avec un traitement adapté (1). Le stade suivant des complications est la péri-implantite. Cette complication est associée à une perte osseuse et n'est pas réversible. La prévalence des péri-implantites est estimée à 10% des implants et 20% des patients au cours des 5/10 ans après la pose de l'implant (2,3).

En général, on observe dans une péri-implantite une perte osseuse marginale en forme de cratère autour de l'implant. Elle s'accompagne la plupart du temps par des manifestations cliniques diverses telles qu'une accumulation de plaque excessive, de saignement au sondage, de récession marginale et une difficulté à mettre en œuvre une hygiène appropriée.

Cette pathologie représente un enjeu de santé publique car l'implantologie est devenue une technique de choix pour remplacer les dents manquantes et les données de l'industrie montrent une augmentation croissante de l'utilisation des dispositifs implantaires. On peut arguer de ces éléments une évolution parallèle du nombre de péri-implantites.

Par ailleurs, si les stratégies de traitement ne montrent pas toutes une efficacité totale (9), différents points caractérisent une péri-implantite. Une évaluation de la totalité des éléments cliniques accompagnant cette pathologie s'avère donc nécessaire pour adopter les décisions thérapeutiques appropriées (4). L'absence de gradation de la maladie ne permet pas de différencier les divers degrés de péri-implantite ainsi que les facteurs de risques associés. Des classifications plus ou moins sommaires ont été publiées au fil des années ; parmi elles, celle de Froum et Rosen proposée en 2012 (5) présentant le même écueil que les autres : une absence de prise en compte de tous les paramètres influant sur la péri-implantite. Il en résulte une confusion dans l'interprétation des données des études évaluant la prévalence, les traitements mis en place et leurs résultats thérapeutiques. Une évaluation standardisée paraît nécessaire pour mettre en œuvre les traitements les plus adaptés en fonction de la

progression de la pathologie. L'objectif de cette étude est de proposer un modèle d'évaluation original du risque de péri-implantite en s'inspirant du modèle d'évaluation du risque parodontal publié par Chandra en 2007 (6).

Le travail constituant cette étude a été rédigé et est actuellement soumis pour publication à la revue : Oral Health and Preventive Dentistry

Manuscript: OHPD-2017-400 - (4639) - Peri-implantitis risk factors: a prospective evaluation

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II. ARTICLE “PERI-IMPLANTITIS RISK FACTORS : A PROSPECTIVE EVALUATION”

Peri-implantitis risk factors: a prospective evaluation

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Abstract

Purpose: This study was carried out with the aim to create a score allowing an evaluation of the risk of peri-implantitis evolution according to its severity and to validate this tool as a new model for peri-implantitis.

Materials et methods: Forty-three patients, 28 of whom were diagnosed with peri-implantitis on at least one implant and 15 patients with implant therapy, were included prospectively in the study in the odontology department of "La Timone" Hospital at Marseille (France). Participants filled in a questionnaire and underwent a thorough clinical examination, a systematic retro-alveolar intra-oral radiography of the implant(s) and a 3D radiography. The following criteria were recorded: the number of implant faces showing bleeding and / or borehole suppuration, the pocket depth on at least two faces of the implant, bone loss as a function of the length of the implant evaluated on X-rays, the number of implant faces with plaque, the parameters required for the determination of excess cement (screwed or sealed prosthesis, burial of sealed prostheses), periodontal status, glycaemia and annual consumption of tobacco. Through Microsoft Excel®, each of these parameters was plotted on a radar chart.

Results: In the proposed evaluation model, 16/28 (57.1%) of cases were identified with high peri-implantitis risk compared to only 2/28 (7.2%) of cases with low risk. The remaining 10/28 (35.7%) correspond to patients with moderate peri-implantitis risk. All patients without peri-implantitis 15/15 (100%) were considered at low risk.

Conclusions: The observed results applied to the proposed evaluation model, constitute an effective diagnostic tool in the peri-implantitis risk assessment. The systematic use of this tool allows an early detection and management of peri-implantitis to improve treatment.

Keywords: Peri-implantitis, risk assessment, evaluation model, prospective

Introduction

When inserting an implant, after obtaining osseointegration, inflammatory complications of infectious origin may appear and affect the peri-implant tissues. The first stage of these complications is mucositis or inflammation of the mucosa without bone loss. Mucositis is reversible with appropriate treatment (1). The next stage of complications is peri-implantitis. This complication is associated with bone loss and is not reversible. The prevalence of peri-implantitis is estimated at 10% of implants and 20% of patients within 5/10 years after implant placement with an increase in prevalence in case of tobacco or old periodontitis (2,3). A more recent study reported a prevalence of 8.8% at 10 years in 20% of patients followed for the evaluation of complications of peri-implant tissues without regular maintenance program (4). Serino observed in his study of partial edentulous patients that peri-implantitis observed were mostly related to accessibility to oral cleaning (5). The prevalence of peri-implantitis correlated with the number of implants inserted in an individual would increase but had no influence on the prevalence of mucositis (6). The peri-implantitis does not affect all patients similarly. Literature reviews observed a significant increase in the prevalence of peri-implantitis in subjects treated for periodontitis (7).

There is no real biological evidence of the disruption of integration nor a description of this phenomenon, Clementini in its systematic review and meta-analysis reveals a multifactorial etiology (8). In general, peri-implantitis has a marginal bone loss in the form of a crater around the implant. It is most often accompanied by various clinical manifestations, such as excessive plaque, bleeding during probing, marginal recession and difficulty in implementing appropriate oral hygiene.

This pathology represents a public health issue because implantology has become a technique of choice to replace lost teeth and industry data (unpublished) show an increase in the use of implant devices. One can argue that these elements are connected to the evolution of the number of peri-implantitis. Moreover, if treatment strategies do not show any total efficiency (9), different points characterize a peri-implantitis. An evaluation of all the clinical elements accompanying this pathology is therefore necessary to adopt the appropriate therapeutic decisions (10). The absence of gradation of the disease does not make it possible to differentiate the various levels of peri-implantitis as well as the associated risk factors. This results in confusion in the interpretation of data from studies evaluating the prevalence, treatment and outcomes of therapeutics (11). A standardized evaluation is needed to implement the most appropriate treatment based on the severity of peri-implantitis whose prognosis remains

uncertain. Moreover, peri-implantitis is a public health problem because the number of implants has been increasing in recent years (unpublished industry data) and probably also the number of peri-implantitis. In this work, we developed a new peri-implantitis evaluation score based on the periodontal risk assessment model (12).

Methods

Model development

A model of periodontal risk assessment (bleeding on probing, prevalence of residual periodontal pockets, loss of teeth, estimation of loss of periodontal support in relation to the patient's age, evaluation of systemic diseases, tobacco habits) was described in 2003 (13). A block diagram taking into account these parameters has been described. This first insufficient approach gave rise to a second functional diagram characterizing more precisely the periodontal score by adding as additional parameters: glycemic status, plaque accumulation, socio-economic factors and stress (12). We developed a new peri-implantitis score based on the one previously reported for periodontitis (12). This score included: bleeding and / or pus on probing, pocket depth, bone loss, excess cement, oral hygiene, history of periodontal disease, tobacco habits and glycemic status.

- **Bleeding and / or pus on probing**

Peri-implantitis is an inflammatory disease affecting soft tissues and hard tissues around an osteo-integrated implant, this why evaluation of the presence of inflammation and bone loss are essential parameters for any diagnostic classification (14). In the classification proposed by Froum and Rosen (11), bleeding and / or pus on probing were mentioned as the best clinical indicators for determining the presence of inflammation. Bleeding can be easily assessed by indicating the presence or absence of bleeding after probing with a colored and graduated periodontal probe or a foam probe with a 0.4 mm diameter end on the 6 faces of the implant. The presence or absence of bleeding is determined by the occurrence of bleeding within 15 seconds following a light probing (15).

- **Pocket depth**

Pocket depth may vary depending on the covering mucosa, the presence or absence of keratinized tissue, the type of restoration limiting the probing and also the pressure applied (16). In addition, it has been established that in the presence of inflammatory tissue, a periodontal

probe penetrates the tissues more deeply (17). Considering that peri-implant probing measurements are more sensitive to variations in force than the probing of periodontal pockets, it was recommended to apply a sampling pressure equivalent to 0.25 N (18).

Instead of using a probe equipped with a pressure sensitive device, a light probing is recommended around the implant. This sampling method, which was recommended to determine pocket depth for this classification, was found to be reproducible to ± 1 mm in more than 95% of cases (19). Excessive probing pressure, even on a complex of healthy soft tissues, can cause fiber breakage and therefore provide false-enhanced sounding mapping. However, a lightweight ≥ 4 mm sound is an appropriate clinical reference in assessing the healthy or pathological status of the peri-implantous mucosa (16). The clinical status of the peri-implant mucosa can therefore be determined by associating depths of probing and bleeding and / or pus with the probing on at least two faces of the implant.

- **Bone loss**

Implants vary in shape and morphology. Numerous studies are based arbitrarily on a bone loss ≥ 1.8 mm (corresponding on average to the third coil of an implant) for the diagnosis of peri-implantitis (20-22). A vertical bone resorption of less than 0.2 mm after the first year of function of an implant is today a generally accepted criterion of success (23). However, accurate assessment of this height is difficult to measure clinically. Therefore, the diagnosis of peri-implantitis based on a crater ≥ 1.8 mm after one year seems difficult to apply by the clinicians (24). Standardized X-ray images can help determine the exact level of bone loss in relation to a fixed reference point (crown-implant junction or implant-abutment for example) but this is difficult to measure and compare in millimeters. The evaluation proposal is therefore based on a comparison of bone resorption over time, determined by changes in the percentage of bone loss relative to the length of the implant.

Following the example of Forum and Rosen's classification (11), several ranges of percentage of bone resorption relative to the length of the implant are found: <10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, > 50%. This facilitates the determination of a distinct change in the severity of a peri-implantitis, from an early to moderate stage, from a moderate to advanced stage. Combined with bleeding and / or pus on probing, the bone loss can diagnose the pathology at its earliest clinically detectable stages and resolve the problem of unnecessary patient overexposure to X-rays. Moreover, the fact that the ratio of peri-implantitis progression / associated bone resorption does not follow a linear curve (acceleration of bone loss over time) underlines the need for early diagnosis and management of pathology (25). Accurate

measurement of the amount of bone resorption around the implants is one of the essential conditions for differentiating the categories of the proposed evaluation model. Several authors emphasize the importance of acquiring a retro-alveolar x-ray on the day of implant insertion with the final prosthesis. An orthopantomogram can hardly replace a retro-alveolar radiography because of deformations preventing any precise comparison. This reference radiography makes it possible to establish the initial ratio between junction between the implant and the pillar (or implant-crown junction) and the first bone-implant contact, making it possible to compare them with subsequent shots in order to determine the "Extent of bone loss at the mesial and distal surfaces of the implant". Removal of the prosthesis during radiographic evaluation may be necessary when it prevents an accurate estimate of the bone level. Probing to the highest level of the buccal and lingual (or palatal) bones after anesthesia of the peri-implant tissues can help to determine the bone levels of these sites, which cannot be verified on a standard retro-alveolar x-ray. This system preserves the bones from the exposure to X-rays. It also avoids the error of bone loss expressed in number exposed turns, which may vary according to the implant system. If the implant exhibits vestibular cortical bone loss due to poor positioning and absence of bleeding at the mucosal level, it is likely that the bone defect is the result of physiological resorption and insufficient vascularization in the vestibular cortical bone (26). Most defects caused by peri-implantitis affect more than one surface. Therefore, determining the level of risk in the proposed classification requires at least a probing depth ≥ 4 mm associated with bleeding and a bone loss on two or more faces of the implant. The mandatory condition to be respected is that the implant must be positioned at a sufficient distance from the vestibular cortical. An implant placed in an excessive vestibular position may result in damaging the alveolar bone and soft tissue not induced by bacteria (27). Thus, the classification category is determined by the most severe deterioration of the implant, all faces combined. In order to avoid classification errors, it is therefore important to measure at least two faces of the evaluated implant.

- **Excessive cement**

Prosthetic cemented crowns on implant represent a common alternative to later-screwed-retained implant crowns. The use of prosthetic cemented crowns on implants frequently results in excessive cement deposits in the peri-implant tissues despite careful clinical control at the time of sealing (28). A further study from Linkevicius (29) showed that the more the cervical limit of a crown is embedded, the greater is the quantity of residual cement. It also underlines the unpredictability of radiographic evaluation in the detection of possible cement excesses. In 4/53 cases (7.5%), cement remains were visible on the mesial surface and in 6/53 cases (11.3%)

on the distal side of the implants. Therefore, dental x-rays cannot be considered as a reliable method of evaluating excessive cementing (29). An excess of cement can be assimilated by a foreign body and thus trigger an inflammatory response leading to a peri-implantitis. Indeed, these cement remains can be the starting point of colonization by oral microorganisms resulting in the appearance of a mucositis and peri-implantitis. Excess cement samples were taken from ten patients to study the formation of the biofilm by molecular method based on 16S rDNA sequencing (30). The results of the *in-situ* hybridization revealed a strong tendency towards the bacterial invasion of methacrylate-based cements. Wilson, using dental endoscopy, found an association between excessive sealing cement and peri-implant pathology in 81% of cases. After suppression of the excesses, clinical and endoscopic signs of peri-implantitis were absent in 74% of the studied implants (31). Although few studies have examined the association between excessive cement and peri-implantitis, the data clearly indicate that an excess of cement can trigger an inflammatory response that causes clinical signs of peri-implantitis / mucositis peri-implant.

- **Oral Hygiene**

As soon as a part of a dental implant is exposed in the oral cavity, a bacterial colonization of this exposed surface occurs (32, 33). In a prospective study, Lindquist associates poor oral hygiene with peri-implant bone resorption (34). From a subsample of 47 initially included in the study (13 patients with good oral hygiene and 14 patients with poor oral hygiene), a 10-year difference was observed. An average bone loss of 0.65 mm was observed in the group with good oral hygiene while 1.65 mm in the group with poor oral hygiene. Inappropriate accessibility to oral hygiene of the implanted sites has also correlated with the peri-implantitis (5). In this study, 48% of implants with peri-implantitis were those that did not allow for proper oral hygiene due to limited access or no access. An analysis of the risk factors of peri-implant pathology by Ferreira *et al.* (35) has allowed poor oral hygiene to be considered a risk factor for peri-implantitis. In a transversal study Roos-Jansåker (36) reported that the presence of dental plaque could explain the manifestation of mucositis peri-implant but not peri-implantitis. A 3-year follow-up study (37) identified dental plaque as a significant factor in the development of peri-implantitis. The accumulation of dental plaque in relation to dental implants is clearly associated with the development of peri-implant mucositis (38), but the latter does not necessarily evolve into peri-implantitis. When this is the case, it has been demonstrated that the onset of the pathology was preconditioned by an infectious process (39-42).

- **Antecedents of Periodontal Diseases**

According to the criteria used to define periodontitis, prevalence ranges from 12% to 76% in the United States (43, 44). Globally, about 10% of the world population has acute periodontal disease. It has been observed that individuals with risk of periodontitis react differently to microbial attacks than patients without risk (45). Individuals with antecedents of periodontal disease may therefore also have an increased risk of developing peri-implantitis. Several studies on the variables of implant success concluded that patients with antecedents of periodontitis had a significantly greater depth probing, marginal bone resorption and peri-implantitis incidence in the long term compared to patients with a healthy periodontium (36, 46-51). Among studies comparing implant survival rates in patients affected and unaffected by periodontitis, a majority demonstrate higher (sometimes twice higher) failure rates in subjects with periodontitis. A recent study using multivariate analysis showed that tobacco consumption ($P = 0.046$) and antecedents of periodontitis ($P = 0.046$) would have an impact on advanced peri-implant bone resorption (52). The presence of periodontal pathogens around failing implants (53-60) could suggest a direct link between periodontitis and peri-implantitis through translocation of these species from their intraoral niches to implant sites as suggested by several studies (40, 32,33). In partially edentulous patients, the teeth act as a reservoir, whereas in the total edentulous patients, the periodontal pathogens remain in the oral cavity, surviving on the tongue or in the saliva (61- 63).

An indirect link may also be considered, especially in patients with aggressive periodontitis who are more prone to peri-implant infections because of their immune response to periodontal pathogens. Thus, several authors report a statistically significant difference in the percentage of peri-implantitis between patients affected and not affected by periodontitis (36, 51, 64-66) with higher values of marginal bone loss around the implants in the periodontitis group (1.3 to 2.0 time more).

Some studies distinguish the patients with moderate or severe periodontitis from those with chronic or aggressive periodontitis. In general, severe / aggressive forms of periodontitis are responsible for the highest rates of peri-implantitis (51,66-69) .

All of these data indicate that patients with a history of periodontal disease are more prone to peri-implantitis, marginal bone resorption around implants, and even implant loss. However, underlying mutual confounding factors, such as tobacco consumption, may partly influence this association (46-50, 70, 71).

While periodontitis can be considered as a plaque-related disease, patients with aggressive periodontitis show certain specificities: an absence of correlation between microbial deposits

and severity of periodontal degradation, high proportions of *Porphyromonas gingivalis* and *Actinomices actinomycetemcomitans*, phagocytosis abnormalities, and macrophage hyperactivity, including elevated levels of prostaglandin E2 and interleukin IL-1β (72, 73). This type of patient also has a polymorphism of genes involved in the coding of many inflammation regulators including IL-1 and TNF (73-75). These polymorphisms result in an altered inflammatory profile, particularly characterized by trans-endothelial migration and signaling by neutrophilic polynuclear cells (76), a reduced chemotactic response, and a decrease in phagocytosis and superoxide anion production by neutrophils (77).

In other words, in patients with chronic periodontitis, proper dental plaque control and smoking cessation will reduce the risk of recurrence of periodontitis, but also the risk of peri-implantitis. These risk factors also affect patients with an antecedent of aggressive periodontitis, making them more susceptible to peri-implantitis. Monje calculated that the ratio of the risk of implant failure in patients with aggressive periodontitis was significantly higher compared to patients without a history of periodontitis (78) and those with chronic periodontitis (79, 80).

- **Tobacco smoking**

The consumption of tobacco alters various aspects of innate and adaptive host immune responses (81-83). In fact, smokers show an increase in granulocyte count and total white blood cell count (84), an increase in the lifetime of polymorphonuclear cells (85), production of superoxide anion and hydrogen peroxide, the expression of integrins (83) and the production of protease inhibitors (86). The humoral immune response is also disrupted by tobacco addiction (87, 88). Smoking has been shown to inhibit the proliferation and / or function of B and T lymphocytes (89). Nicotine is the most widely studied component of tobacco for its effects on various cell populations of the periodontium. Nicotine concentrations in smokers' cervical gingival fluid, were nearly 300-fold higher than those found in plasma (20 ng/ml) (81). Gingival bleeding and cervical fluid flow increases from 3 to 5 days after smoking cessation (90). This finding, coupled with the finding of post-weaning bleeding increase (91), confirms the suppressive impact of chronic tobacco consumption on gingival fluid flow and vascularization. Several studies have shown that nicotine has properties that can alter healing (92-96).

Studies comparing the subgingival microbiota of smokers and non-smokers have yielded contradictory results (79, 80, 97). Several studies have associated the smoking with periodontitis, dental loss, alveolitis, and altered healing after surgery (98-104).

Data obtained by the studies evaluating the impact of smoking on implant survival clearly demonstrate the negative effect of tobacco consumption. They suggest that individuals who

smoke before or after implantation may be at risk of implant failure between 35% and 70% higher compared to non-smokers. This affirmation is in line with several systematic reviews of the literature (105-108). There were no statistically significant differences in the complications between non-smokers and former smokers (109), indicating that the risk of complications may be reduced to a "normal rate of complication as in the non-smoker" when the tobacco consumption stops. Several prospective clinical trials, with a considerable follow-up time (often more than 5 years), revealed differences in the incidence of peri-implantitis between smokers and non-smokers/ex-smokers.

Although the relationship between smoking and peri-implantitis remains controversial, several studies have reported a statistically significant difference in peri-implantitis incidence between smokers and non-smokers (36, 110), with strong association between peri-implantitis and smoking (111).

- **Diabetes**

The association between periodontal diseases and systemic pathologies has aroused great interest during the last decade. The Workshop of the European Federation of Periodontology and the American Academy of Periodontology on Periodontitis and General Diseases concluded that "there is solid and consistent epidemiological evidence that periodontitis predisposes to increased risk of Cardiovascular disease » (112); "Severe periodontal disease affects the glycemic control of diabetics and influences glycemic levels in non-diabetic patients. In diabetic patients, there is a direct and dose-dependent relationship between the severity of periodontitis and diabetes complications" (113). Based on available data on the association between periodontal disease and systemic pathologies, it seems logical to assume that these pathologies may also have an impact on the development of peri-implantitis. Ferreira demonstrates, through univariate analysis, that subjects with diabetes are more likely to develop peri-implantitis (35). This trend is confirmed by the Gomez-Moreno study: in patients with type 2 diabetes with a follow-up over a three-year period, marginal bone loss and bleeding at the peri-implantation level correlated with the increase of the level of glycated hemoglobin A1c (HbA1C) (114). However, in his recent study, Aguilar-Salvatierra finds that patients with type 2 diabetes can receive implant treatments with immediate safe loading provided they have moderate HbA1c levels (115). Although data on possible associations between systemic diseases and peri-implantitis remain limited up to date, there are certainly relationships between peri-implant disease and diabetes. New studies should be conducted to confirm these associations on larger populations of patients.

Calculation method

- **Bleeding and / or pus on probing**

The method of Ainamo *et al.* (15) cited by Froum and Rosen in 2012 (11) served as a reference. Bleeding on probing can be easily evaluated by indicating the presence or absence of bleeding after probing with a probe on the 6 faces of the implant. The presence or absence of bleeding on probing is determined by the occurrence of bleeding within 15 seconds following a light probing. It is realized on the six sides of the implant: mesio-vestibular, vestibular, disto-vestibular, mesio-lingual/mesio-palatal, lingual/palatal, disto-lingual/disto-palatal, measured after 15 seconds and expressed in number of affected implant faces (0 to 6) **(Table 1**).

- **Pocket depth**

Considering that peri-implant probing measurements are more sensitive to variations in force than the sounding of periodontal pockets, it is recommended to apply a sample pressure equal to 0.25 N (18). The around implant soft clinical probing, recommended to determine the pocket depth for this classification, was found to be reproducible to ± 1 mm in more than 95% of cases (19). In order to have reproducibility, a probing force of 0.25 N is applied using a graduated periodontal probe. This probing is carried out on at least two faces of the implant and is expressed in millimeters (**Table 1**).

- **Bone loss**

Bone loss is evaluated from retro alveolar intraoral radiography (assessment of the mesial and distal crater), it is expressed as a percentage of bone resorption relative to the length of the implant (Table 1). One possibility is the use of 3D imaging with in particular the Cone Beam which would allow a volume measurement of the bone crater. This device is more and more often present in dental offices and offers a high precision for a lower quantity of x-ray compared to a scanner.

- **Antecedents of Periodontal Diseases**

The score is calculated according to the periodontal status (healthy / treated / pathological) and the type or stage of the periodontal disease (**Table 2**).

- **Glycemic Status**

In the evaluation model proposed by Chandra (12), the selected parameter is fasting glycaemia. It is expressed in mg/dl. Thus, according to the diagnostic reference values established by the

American Academy of Periodontology (116), a glycaemia value of less than 110 mg/dl implies a low periodontal risk and a value greater than 126 mg/dl, a high risk. Any value between these two values reflects a moderate risk. However, glycaemia provides only a snapshot of glycemic status, while glycated hemoglobin A1c (HbA1c) is useful in assessing glycemic control over a longer period of time (approximately two to three months). It is therefore a more interesting constant ; It is a marker of the risk of long-term complications of diabetes (Table 2).

- **Tobacco smoking**

Referring to earlier models such as Renvert and Persson, the number of cigarette packets per year served as a reference for the study (117). Considering that the number of cigarettes per packet varies between countries, this can be a confounding factor (118). Like Chandra's evaluation model (12), this criterion is now measured in cigarettes per day, in order to take this possibility into account (Table 2).

- **Oral hygiene**

Hygiene is measured by the presence or absence of bacterial subgingival plaque on the 4 faces of the implant: mesial, distal, vestibular and palatal or lingual. It is expressed in number of implant surfaces affected (0 to 4); for implant-supported prostheses (Brånemark prostheses, implant-supported bridges), a further assessment of the accessibility to hygiene is proposed (yes/no): “yes” if the patient can brush in the implanted region using a toothbrush, little brush or dental floss, “no” in case of limited access (linked to the emergence profile in particular) and / or disability of the patient **(Table 2**).

- **Excessive cement**

Considering the feature of radiographic evaluation in the detection of excessive cement, the calculation of this parameter is essentially clinical. The exact amount of residual cement is impossible to measure accurately, therefore the correlation between burying the cervical boundary of a sealed crown and the amount of residual cement is used. Indeed, Linkevicius has demonstrated that the more this limit is below the gingiva, the greater is the quantity of cement in excess (29). Therefore, the following parameters have been chosen: the determination of the implant abutment / prosthesis assembly means (screwed prosthesis or a sealed prosthesis); in the case of a sealed prosthesis, the measurement of the relative position of the cervical limit with respect to the marginal gingiva (**Table 3**).

The developed model

From a graphical point of view, the peri-implantitis risk analysis model is similar to Chandra's model of periodontitis evaluation (12). It generates a functional diagram which, according to the limits of the polygon obtained, allows to classify a patient into 3 categories: low, moderate or high risk. Indeed, the risk is evaluated by means of a color scale ranging from 0 to 3 (**Figure 1**).

- **Low risk of peri-implantitis**

A peri-implantitis risk is considered to be low if all the parameters are in the low-risk area or if a maximum of two parameters are in the moderate-risk zone and the high-risk area (**Figure 2**). For example, the patient with low peri-implantitis risk presented 3 out of 6 implant faces with bleeding on probing, a pocket depth < 4 mm on at least 2 faces of the implant, a bone resorption < 30% of implant length (screwed prosthesis), 2 implant faces with plaque, severe chronic periodontitis, and non-smoker and non-diabetic.

- **Moderate risk of peri-implantitis**

A risk of peri-implantitis is considered to be moderate if at least three parameters are in the moderate risk zone and no more than one parameter is in the high-risk area (**Figure 3**). For example, the patient at moderate risk of peri-implantitis had 3 implant faces on 6 with bleeding on probing, a pocket depth ≥ 6 mm on at least 2 faces of the implant, bone resorption > 30 % of implant length, sealed prosthesis with limit bury under the gingiva < 1 mm, 2 implant faces with plaque, chronic periodontitis treated in 2008 and non-smoker and non-diabetic.

- **High peri-implantitis risk**

A risk of peri-implantitis is considered high if at least 2 parameters are in the high-risk area (**Figure 4**). For example, the patient at high peri-implantitis risk presented suppuration on all implant surfaces, a pocket depth ≥ 8 mm on at least 2 faces of the implant, bone resorption > 50% of implant length, a screwed prosthesis with intra-sulcus margin of 1 to 2 mm, dental plaque on 1 implant surface, severe chronic periodontitis, was non-smoker and diabetic with HbA1c > 8%.

Evaluation of the severity analysis model

Twenty-eight patients diagnosed with peri-implantitis in at least one implant and 15 patients without peri-implantitis were randomly selected from the odontology department at “La Timone” Hospital in Marseille, France. For each of them, an informed consent form and a questionnaire were obtained. This study is a prerequisite for the approval of the ethics committee of the University Hospital Institute (IHU) in Marseille under number 2016-011. A thorough clinical examination was performed and a retro-alveolar intraoral radiography of the implant(s) with peri-implantitis was also performed. In addition, patients with peri-implantitis were subjected to a 3D radiography of the Cone Bean CBCT type in order to complete the radiological evaluation. In order to avoid errors due to observer variability, all clinical and radiographic examinations were carried out by two previously trained dental surgeons. The parameters recorded were: the number of implant faces with bleeding and / or suppuration on probing, the pocket depth on at least two faces of the implant, the bone loss relative to the implant length evaluated on dental x-rays, the number of implant surfaces with plaque, the parameters required for the determination of excessive cement (screwed or sealed prosthesis, bury limit of sealed prosthesis), periodontal status, diabetes and tobacco consumption. With Microsoft Excel® software, each of these parameters has been plotted on a radar chart. The proposed model could thus be applied to the cohort of patients with peri-implantitis and controls. The results obtained made it possible to evaluate the risk specific to each patient presenting with peri-implantitis.

Results

Sixteen male and twenty-seven female patients aged 35-86 with an average age of 62.7 years were enrolled in the study: 28 patients with peri-implantitis and 15 patients without peri-implantitis. All patients received implant therapy. Three peri-implantitis cases were found in the same patient. In total, in individuals with peri-implantitis, 10 had generalized bleeding and 18 had localized bleeding. Pocket depths ranged from 4 to 12 mm. The number of implants affected by the peri-implantitis varied from 1 to 7. In 4 cases of peri-implantitis, the presence of pus was observed. Vertical resorption of the carrier bone (% of implant length) ranged from 29 to 100% (100% in only one case) (**Table 4**). In addition, 4 individuals were smokers and two other patients were former smokers. One patient was confirmed diabetic. Finally, 22 individuals had active chronic periodontitis, including a debilitating form, four moderate and 17 severe forms. Of the remaining six patients, 3 had a history of treated and stabilized chronic periodontitis. The last 3 showed no signs of periodontal disease.

Of the 28 patients with peri-implantitis, 25 had one (or several) peri-implantitis(s) associated with an implant-supported crown or implant-supported bridge. Thirteen prosthetic elements were assembled by sealing, and twelve screwed. The remaining three patients had complete "Bridge Brånemark" prostheses.

In the proposed evaluation model, 2 cases with low peri-implantitis risk were identified, while 10 moderate-risk and 16 high-risk cases were identified. Approximately 57.1% of cases are in the high-risk peri-implantitis category while only 7.2% of cases are in the low-risk category. The remaining 35.7% correspond to patients with moderate peri-implantitis risk (**Table 5**).

Among the control patients, 7 presented with localized bleeding, 2 with generalized hemorrhage and 6 patients showed no bleeding. Pocket depths ranged from 1 to 3 mm with the exception of one patient with a pocket which measured 4 mm. The presence of bacterial plaque was observed in 7 patients on 25% of the surfaces of the implants. No pus was found in any case.

Discussion

This study was conducted to develop a risk assessment of peri-implantitis, the proposed model is based on an already published effective model evaluating periodontal risk (12). This previous model has proved its usefulness and allowed to evaluate precisely the periodontitis. The model developed was tested on 28 patients with a peri-implantitis and 15 patients with no peri-implantitis. This work allowed to classify patients with peri-implantitis in three categories, each corresponding to a level of risk of evolution of peri-implantitis (low: 2/28 (7.2%), moderate: 10/28 (35,7%), high: 16/28 (57.1%)) All 15 control patients were classified in the low risk area, which validates the model developed.

The obtained score provides quantitative information to the clinicians, while the polygon from the functional diagram adds a qualitative component to the diagnosis. The advantage of the proposed model is therefore double: it is a tool to aid diagnosis while standardizing individual care. After reviewing the literature, eight parameters were selected to be part of the proposed evaluation model; however, several criteria, notably of a clinical nature, were not included. Among them, occlusion and the amount of keratinized mucosa must be mentioned. Concerning the occlusal factor, a biomechanical overload at the bone-implant interface could be the cause, or at least contribute to a marginal bone loss according to the hypothesis of occlusal surcharge. Some recent studies consider that occlusal load / occlusion could be a contributing factor to peri-implantitis (119, 120) But the question remains open and this is the reason for not including this parameter into this model. However, although evidence of the impact of occlusal surcharge on peri-implantitis is lacking, an assessment of patient occlusion during maintenance visits seems desirable (121, 122).

The presence of keratinized gingiva and its required level of thickness in the peri-implant region has little influence as far as proper oral hygiene is maintained. Contrary to the keratinized mucosa which when absent can lead to difficulty in accessing and / or a sensitivity to brushing which impairs the control of the dental plaque and leads to important tissue lesions. For cases with a keratinized gingiva defect in the proximity of the implant, the study concludes that the lack / absence of tissue does not necessarily negatively affect the health of the peri-implant tissues. In the event that the absence of keratinized gingiva would interfere with the control of the elimination of the dental plaque, this proves to be an indirect impact parameter. According to the proposed model, it makes more sense to include a direct assessment of oral hygiene, rather than a clinical criterion influencing it. Despite the fact that this parameter is not included in the evaluation model, the question of the importance of keratinized mucosa at the peri-

implant level persists and this factor should be discussed in future studies on the incidence of peri-implant diseases.

The genotype and its association with tobacco consumption were not included in this model. According to the literature, the polymorphism of the IL-1 gene would have an impact on the peri-implantitis, especially in the case of heavy smokers. Feloutzis *et al*. (123) reported significant differences in alveolar bone loss between non-smokers and heavy smokers in the "IL-1 positive genotype" group and none in the "IL-1 negative genotype" group. Gruica (124) showed that heavy smokers with positive IL-1A and IL-1B genotypes had a higher risk of developing inflammatory complications and increased peri-implantous marginal bone resorption compared to non-smokers of the same genotype. A latest study by Laine *et al.* (125) found an association between the polymorphism of the IL-1RN gene and peri-implantitis, particularly in smokers. The proposed evaluation model is based on a simple, rapid and reproducible measurement of each parameter, while remaining accessible to the clinician in his everyday practice. Given the multiple limitations associated with a genotype study, it was decided not to include this criterion into the evaluation model.

It is possible to compare the proposed evaluation model with the work of Froum and Rosen (11) on a new classification of peri-implantitis. Froum classifies the severity of peri-implantitis based on three clinical criteria: bleeding and / or probing suppuration, pocket depth, and peri-implant bone resorption level. The objective of his work is to propose a standardized classification that can be used for communication between researchers and clinicians, allowing a better comparison of the results and calibration of the studies. This classification is limited because it defines an implant status by only three criteria whereas our proposed model allows us to define a risk of progression of the peri-implantitis basing on eight parameters calibrated between them. While Forum measures the severity of the pathology at a specific time, our proposed evaluation model assesses its susceptibility to evolution. The comparison of the results of this study with that of Froum and Rosen makes it possible to demonstrate a link between the severity of a peri-implantitis and its susceptibility to aggravation: the more severe the peri-implantitis is, the greater is the risk of progression. On the contrary, the earlier the diagnosis of peri-implantitis is established, the less likely it is to develop. This correlation confirms the non-linear evolution of peri-implant pathologies, a fortiori if aggravating factors are added (diabetes, tobacco consumption, periodontal disease, residual cement, poor oral hygiene) as shown by the qualitative analysis allowed by the proposed functional diagram (**Figure 1**).

The results of this study highlight one singularity: the low percentage of patients with low peri-implantitis risk 2/28 (7,2%). This reflects the difficulty in diagnosing early forms, which are often asymptomatic with little or no clinical manifestations and are most often the object of fortuitous discoveries. Moreover, the therapeutics is all the more effective as the management of the peri-implantitis is precocious (25). As Serino and Turri demonstrated in their study (126), the proportion of unhealthy implants returned healthy two years after surgical treatment was higher for implants with a smaller initial bone crater (2-4 mm) compared to implants with initial bone loss ≥ 5 mm (respectively 74% and 40%).

From early diagnosis to regular maintenance, to the rapidity of treatments, prevention has an essential role in the management of peri-implantitis and in reducing their occurrence (9). It is also important to underline the multifactorial aspect of this pathology, hence the importance of an overall understanding of the parameters influencing the level of peri-implantitis risk. The ultimate aim is to categorize each patient and to adapt the therapy individually according to the level of risk of each one.

In order to ensure that this work can serve all students, practitioners and research community the diagnostic tool is available at the following address: http://diagnostic-tool.pagesperso-orange.fr/.

The calculation rules are the same as those described in this article. This online availability is part of the purpose of screening and early care to enhance the outcome of peri-implantitis treatment.

Table and figure references

Table 1: Coding system for bleeding on probing and/or suppuration, probing depth and bone loss

Coding system for bleeding on probing / presence of pus, probing depth and bone loss			
Axis score	Faces with bleeding on probing/presence of pus	Probing depth on at least 2 implant faces (mm)	Bone loss related to the implant length (%)
0	0	< 4	< 10
1	1	≥ 4	10 - 20
2	2	≥ 5	20 - 30
3	3	≥ 6	30 - 40
4	4	≥ 7	40 - 50
5	5 - 6	≥ 8	> 50

Table 2: Coding system for periodontal diseases, glycemic status, smoking and oral hygiene

Coding system for periodontal diseases, glycemic status, smoking and oral hygiene				
Axis Score	Periodontal status	HbA1c level (%)	Smoking (cigarettes/day)	No. of implant faces with presence of plaque
0	Healthy periodontium	≤ 6	Non-smoker	0
1	Treated periodontitis	6,1 - 7	Former-smoker	1
2	Slight chronic periodontitis	7,1 - 8	< 10	2
3	Moderate chronic periodontitis	8,1 - 9	10 - 19	3
4	Severe chronic periodontitis	9,1 - 10	20	4
5	Aggressive periodontitis	> 10	> 20	No accessibility to oral hygiene

Table 3: Coding system for excessive cement according to assembly means and cervical margins position.

Coding system for excessive cement according to assembly means and cervical margins position	
Axis score	
0	Screw-retained prosthesis
1	Cemented prosthesis with supragingival cervical margin
2	Cemented prosthesis with juxtagingival cervical margin
3	Cemented prosthesis with intra-sulcus margin < 1 mm
4	Cemented prosthesis with intra-sulcus margin of 1 to 2 mm
5	Cemented prosthesis with intra-sulcus margin > 2 mm

Table 4: Clinical data collection of pathological implants (P) and controls (C): sex, with bleeding on probing (BOP), probing depth (PD), surfaces with presence of plaque (%), presence of pus, number of implants affected by peri-implantitis, mobility, vertical resorption of the supporting bone (% of implant length).

Sample	Sex	BOP	PD (mm)	Surfaces with presence of plaque (%)	Presence of pus	Number of implants affected by peri-implantitis	Presence of mobility	Vertical resorption of the supporting bone (% of implant lenght)
P1	f	Generalized	8	50	No	4	No	48
P2	f	Generalized	7	100	Yes	2	No	70
P3	f	Generalized	7	25	No	1	No	51
P4	m	Localized	4	100	No	2	No	37
P5	m	Localized	6	100	No	7	No	46
P6	f	Localized	4	50	No	2	Yes	29
P7	f	Generalized	7	50	No	3	Yes	69
P8	f	Localized	8	25	Yes	1	No	51
P9	f	Localized	5	25	No	2	No	64
P10	m	Localized	4	25	No	1	No	46
P11	f	Localized	7	100	Yes	2	No	71
P12	f	Localized	12	25	No	1	Yes	100
P13	f	Localized	10	50	No	5	No	60
P14	f	Generalized	9	100	No	2	Yes	74
P15	f	Localized	6	100	No	1	No	52
P16	m	Generalized	5	100	Yes	6	No	35
P17	f	Localized	7	25	Yes	7	No	67
P18	f	Localized	8	100	Yes	1	No	60
P19	m	Generalized	7	100	Yes	4	No	57
P20	f	Localized	10	100	Yes	2	No	81
P21	f	Localized	6	50	No	1	No	50
P22	m	Localized	6	100	Yes	1	No	35
P23	f	Localized	9	100	No	2	No	40
P24, P25, P26	f	Generalized	10	100	No	4	No	85
P27	m	Localized	6	100	No	2	No	36
P28	m	Generalized	7	100	Yes	2	No	35
P29	f	Generalized	9	50	No	4	No	46
P30	f	Localized	6	50	No	2	No	35
C1	m	Localized	1	25	No	0	No	4
C2	f	Ø	4	0	No	0	No	15
C3	f	Localized	1	25	No	0	No	9
C4	f	Ø	2	0	No	0	No	11
C5	f	Localized	1	0	No	0	No	17
C6	m	Generalized	1	0	No	0	No	8
C7	f	Localized	3	0	No	0	No	9
C8	m	Localized	1	25	No	0	No	11
C9	f	Ø	3	0	No	0	No	9
C10	m	Ø	1	25	No	0	No	2
C11	m	Localized	1	25	No	0	No	5
C12	m	Generalized	1	25	No	0	No	6
C13	m	Ø	1	0	No	0	No	16
C14	m	Ø	1	0	No	0	No	2
C15	f	Localized	1	25	No	0	No	0

Table 5. Distribution of low, moderate and high-risk cases according to the proposed assessment model.

Distribution of low, moderate and high-risk cases according to the proposed model			
Risk level			Total
Low risk	Moderate risk	High risk	
2 (7,2%)	10 (35,7%)	16 (57,1%)	28 (100%)

Figure 1: Proposed risk diagram with the clinical criteria, radiographic measures and risk factors divided in eight parameters on three separate risk areas: low, moderate and high.

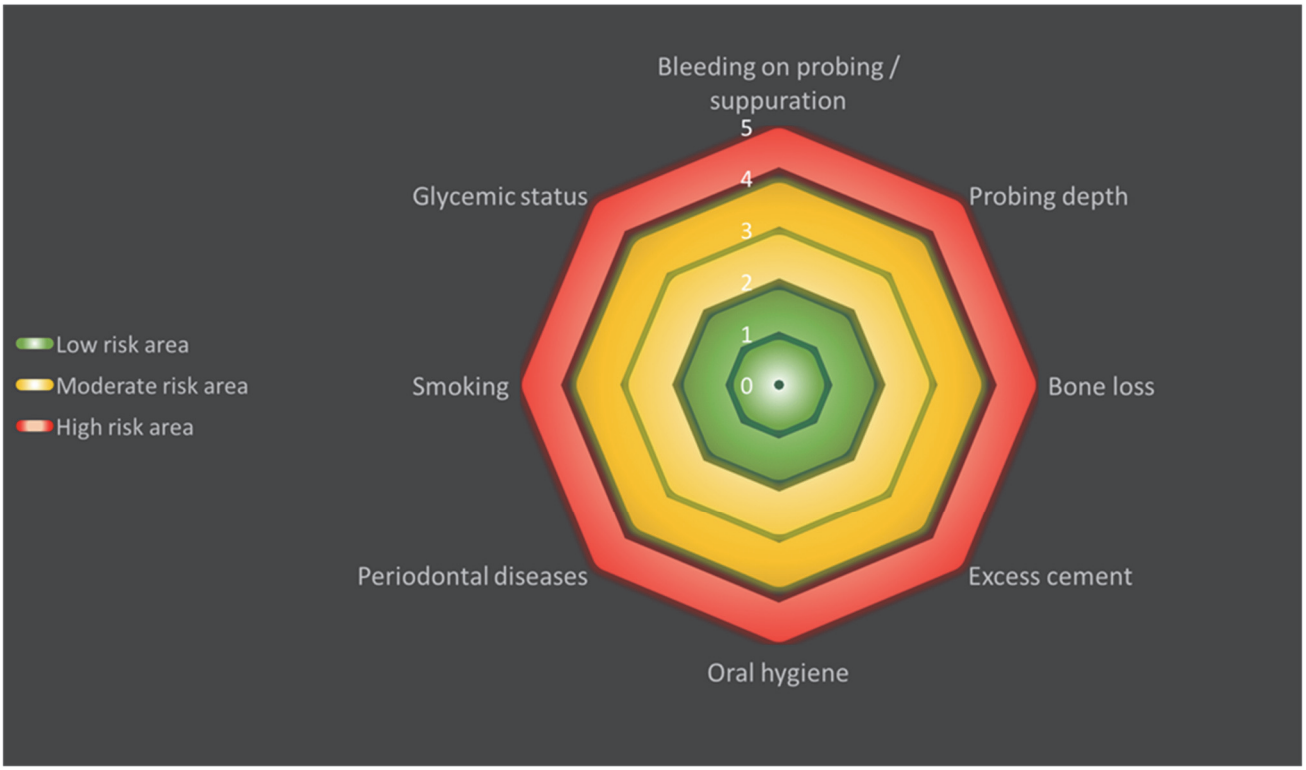


Figure 2: Patient with low peri-implantitis risk: 3 out of 6 implant faces with bleeding on probing, a probing depth < 4 mm on at least 2 faces of the implant, a bone resorption < 30% of the implant length (screwed prosthesis), 2 implant faces with plaque, severe chronic periodontitis, and non-smoker and non-diabetic.

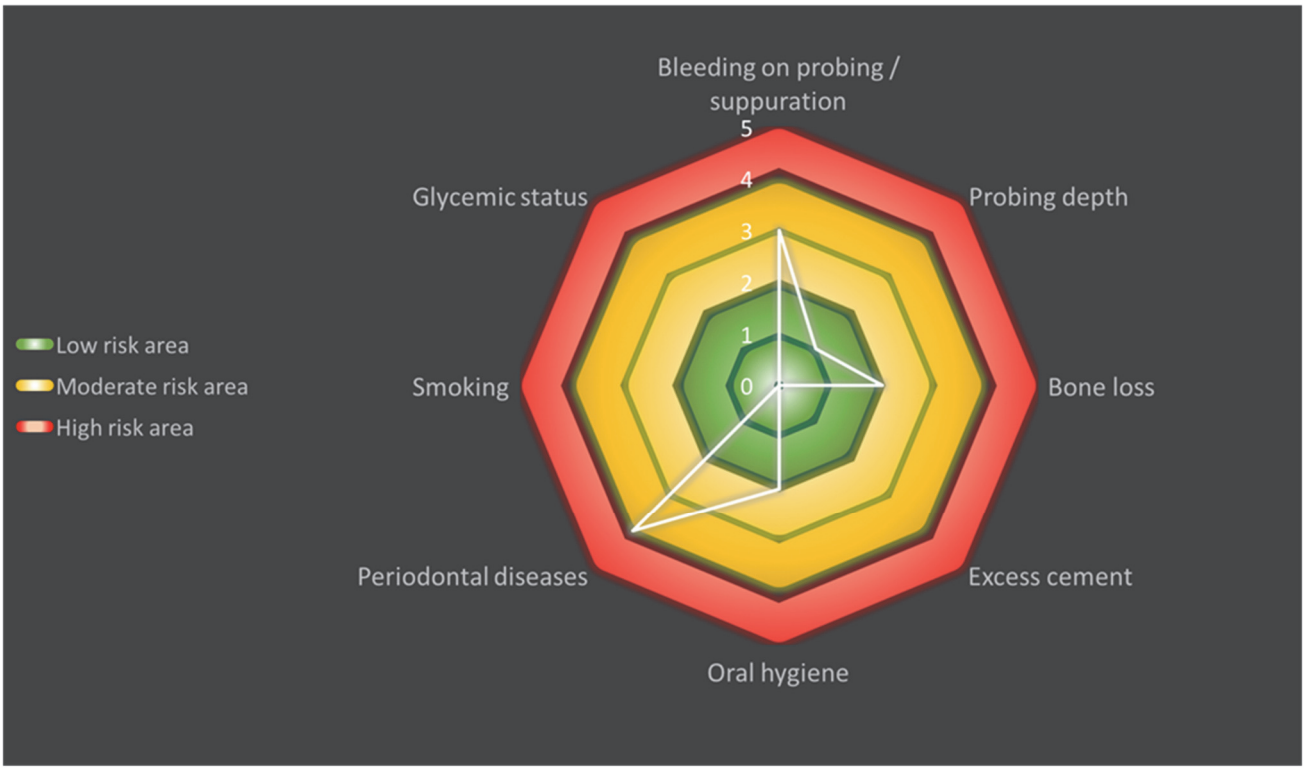


Figure 3: Patient with moderate peri-implantitis risk: 3 implant faces on 6 with bleeding on probing, a probing depth ≥ 6 mm on at least 2 faces of the implant, bone resorption > 30 % implant length, sealed prosthesis with intra-sulcus margin < 1 mm, 2 implant faces with plaque, chronic periodontitis treated in 2008, non-smoker and non-diabetic.

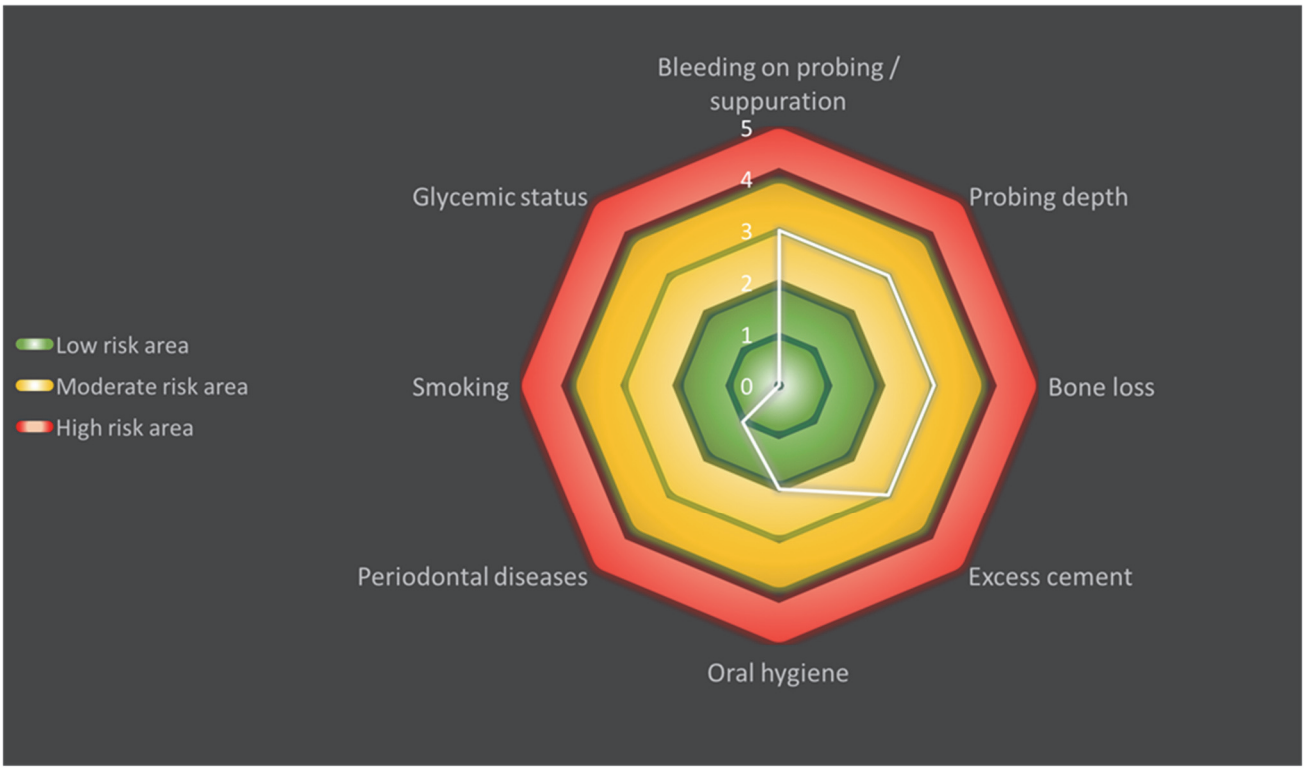
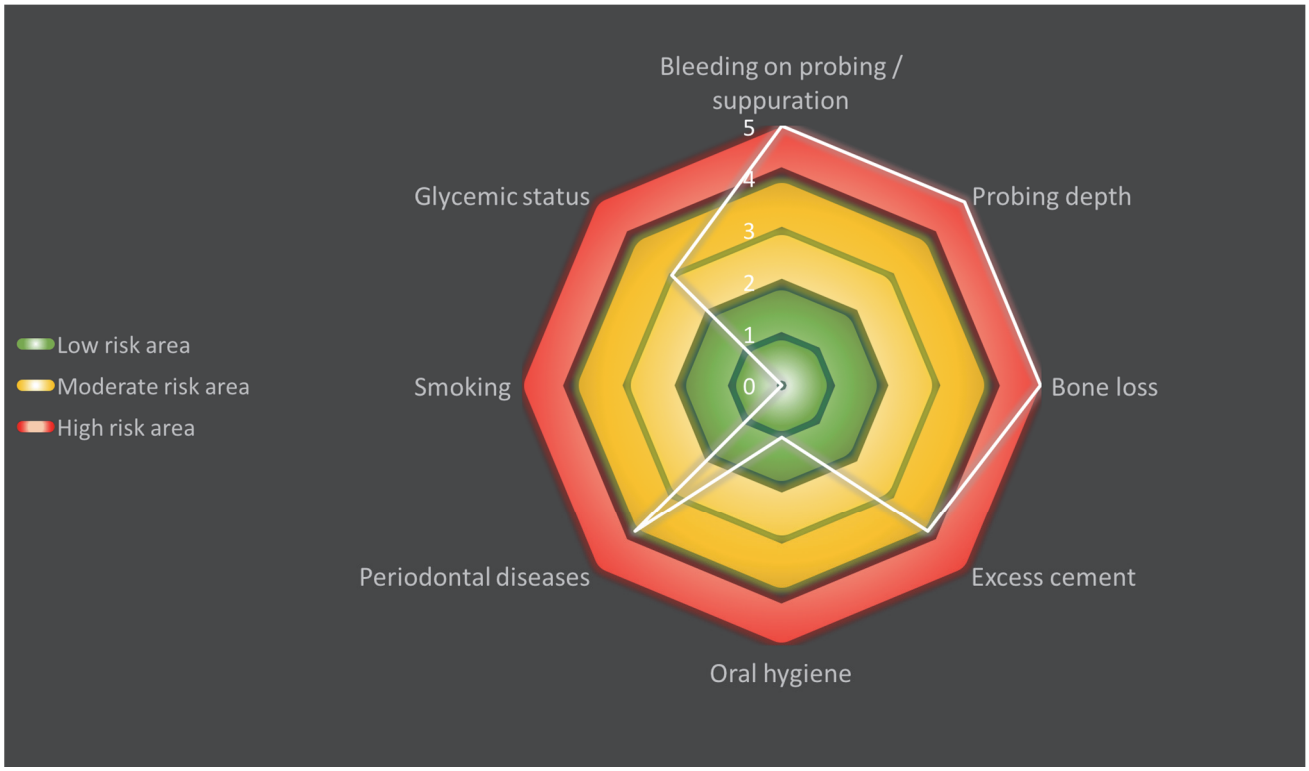


Figure 4: Patient with high peri-implantitis risk: suppuration on all implant surfaces, a probing depth ≥ 8 mm on at least 2 faces of the implant, bone resorption > 50% implant length, a screwed prosthesis with intra-sulcus margin of 1 to 2 mm, dental plaque on 1 implant surface, severe chronic periodontitis, non-smoker and diabetic with HbA1c > 8%.



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III. LA FLORE MICROBIENNE DES PÉRI-IMPLANTITES

Dès lors qu'une péri-implantite est établie, le site est colonisé par des pathogènes. La flore microbienne retrouvée dans les péri-implantites est principalement constituée par des pathogènes anaérobies, notamment des bactéries Gram négatif telles que *Fusobacterium* spp. et *P. intermedia* qui sont régulièrement détectées (25).

Parmi les pathogènes associés aux parodontites, les travaux menés au sein de l'URMITE (Unité de recherche sur les maladies infectieuses et tropicales émergentes) ont montré la présence d'archaea méthanogènes. Les archaea méthanogènes sont des procaryotes anaérobies stricts dont les conditions de culture restent fastidieuses et mal connues. Certaines archaea méthanogènes ont été impliquées dans la parodontite. *Methanobrevibacter oralis* est l'archaea méthanogène dominante dans la cavité orale. Celle-ci a été isolée et formellement identifiée. *M. oralis* a été trouvée dans toutes les études avec une prévalence > 40% alors que les autres archaea ont été identifiées dans certaines études avec une prévalence < 20% et même < 10% (7,8,9,10). *M. oralis* a été isolée initialement à partir de la plaque dentaire de sujets sains (11) et ultérieurement à partir des patients atteints de parodontite (12). Plus récemment une nouvelle espèce d'archaea méthanogène a été isolée à partir de prélèvements de poches parodontales. Cette archaea a été nommée *Methanobrevibacter massiliense* (12). Par ailleurs, *M. oralis* a été détectée également dans des sites de péri-implantite et dans des infections endodontiques (9,13). Cependant, les études menées n'ont pour l'instant pas pu mettre en évidence l'implication relative de ces espèces dans la pathogénèse des péri-implantites (9). L'objectif du second travail a été d'établir le répertoire des archaea méthanogènes des péri-implantites. Pour cela, dans le cadre de ma thèse, j'ai effectué des prélèvements de poches de péri-implantites lors des relevés des données cliniques permettant de proposer une classification des péri-implantites. Cette étude a fait l'objet d'une approbation du comité d'éthique de l'Institut Hospitalo-Universitaire ; les patients ayant reçu une information détaillée de l'étude ont approuvé par un consentement éclairé le prélèvement.

Ces prélèvements étaient destinés à une analyse moléculaire microbiologique et en particulier la recherche des archaea méthanogènes afin d'établir le répertoire de celles-ci dans des poches de péri-implantites et de dents saines.

En associant cette étude microbiologique à la première étude clinique, l'objectif était également d'établir une corrélation entre la présence et la concentration des espèces d'archaea méthanogènes d'une part, et le niveau de risque péri-implantaire fourni par le modèle proposé afin de valider ce dernier en tant qu'outil diagnostic d'évaluation du risque péri-implantaire.

L'analyse moléculaire a été effectuée par mademoiselle Souad Belkacemi dans le cadre de son stage pratique de master 2. Le stage pratique a été réalisé dans l'URMITE Méditerranée Infection Marseille sous la direction du Professeur Michel Drancourt et du Docteur Gérard Aboudharam.

Le travail constituant cette étude a été rédigé et soumis pour publication à la revue : Archaea. Il a fait l'objet d'une revue et la version révisée a été soumise le 24-08-2017.

Dear Dr. Drancourt,

The Research Article titled "Peri-implantitis-associated methanogens: a preliminary report," by Souad Belkacemi, Anthony Mazel, Delphine Tardivo, Patrick Tavitian, Grégory Stephan, Giancarlo Bianca, Elodie Terrer, Michel Drancourt and Gérard Aboudharam has been received and assigned the number 5734598.

All authors will receive a copy of all the correspondences regarding this manuscript.

Thank you for submitting your work to Archaea.

Best regards,

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****
Yasmine Nasr
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IV. ARTICLE "PERI-IMPLANTITIS-ASSOCIATED METHANOGENS: A PRELIMINARY REPORT"

Peri-implantitis-associated methanogens: a preliminary report

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Keywords:

Peri-implantitis, methanogenic archaea, *Methanobrevibacter oralis*, *Methanobrevibacter masilliense*.

Abstract

Methanogenic archaea (methanogens) have already been described in periodontitis. The aim of this study was to describe the repertoire of methanogens related to peri-implantitis, their prevalence and diversity in samples of peri-implantitis and healthy control teeth and to assess whether methanogens were involved in peri-implantitis. Twenty-eight consenting patients with peri-implantitis were recruited. Thirty samples were collected from peri-implantitis sites and 28 samples were collected from the sulcus healthy teeth of the same patients, constituting the control group. These 58 samples were analyzed by PCR targeting the archaeal 16S rRNA gene and the gene *mcr*A specific to methanogens. Direct sequencing showed that the methanogen community was dominated by *Methanobrevibacter oralis* found in 31/58 (51%) samples including 16/28 (57%) control samples and 15/30 (50%) peri-implantitis samples. Further, "Methanobrevibacter *massiliense"* was detected in 5/58 (8.6%) samples including 3/28 (1%) control samples and 2/30 (6.7%) peri-implantitis samples. For methanogenic archaea PCR was positive in 62% (36/58) of all the samples; 67,8% (19/28) for the control group and 56% (17/30) for the peri-implantitis group. The prevalence of *M. oralis* "*M. massiliense"* was not significantly different in the peri-implantitis samples and in control samples (exact Fisher test, P=0.61 and P= 0.67, respectively). Further ponderation of the methanogen load by the real-time quantitative PCR for actin human gene again indicated non-significant difference (Wilcoxon-Mann-Whitney test, P= 0.48 and P= 0.40, respectively). These data show that the prevalence of methanogens does not differ in peri-implantitis lesions and healthy sites, when individuals are their own control. These data do not allow assigning a specific pathogenic role to methanogens in peri-implantitis; methanogens rather are part of the commensal and normal flora of the oral cavity.

Introduction

Peri-implantitis is an inflammatory disease characterized by the destruction of soft and hard tissues and the formation of pockets around osteo-integrated dental implants [1]. Contrasting with metagenomics studies which failed to detect any methanogen in peri-implantitis lesions [2, 3], methanogen-targeting molecular investigations detected DNA sequences specific for the methanogens *Methanobrevibacter oralis*, *Methanobrevibacter smithii, Methanomassiliicoccus luminyensis*, "*Methanobacterium congolense/curvum"* and "*Methanobrevibacter massiliense"* in periodontitis pockets [4-9]. "*M. oralis"*, an oral cavity inhabitant [10] has been more frequently found in periodontitis pockets [9] in association with anaerobes [11]. Yet, studies relying on the simple, un-quantified molecular detection of sequences leaved unsatisfied the relative role of these methanogens in the pathogenesis of peri-implantitis [8]. We hypothesized that the relative load of methanogens among the total flora in peri-implantitis lesions, would give an indication on the relative role of methanogens in the pathogenesis of peri-implantitis as previously showed for periodontitis [12].

Patients and methods

Twenty-eight patients diagnosed with peri-implantitis were prospectively included in this study from January 2016 to June 2016. This study was approved by the clinical research ethics committee of the IFR 48 of the University of Aix-Marseille approved (protocol N° 2016-011). The study protocol was explained to each patient and all patients signed an information document and an informed consent document. All patients were interviewed in order to collect their medical and dental history. An intra-oral examination was performed to measure bleeding on probing (BOP), probing depth (PD), the surfaces with presence of plaque (%), the presence of pus, the number of implants affected by the peri-implantitis, the presence of mobility and the vertical resorption of the supporting bone; in addition, a radiographic status and a 3D radiography were realized. Subgingival dental plaque samples were collected from all peri-implantitis pockets of each individual with sterile Gracey curettes 1/2 (Hu-Friedy, Rotterdam, Netherlands) and placed into tubes containing 5 mL of the SAB anoxic transport medium for methanogens composed of 0.1g/L; MgCl, 0.2 g/L; KH_2PO_4, 0.2 g/L; KCl, 0.1 g/L; $CaCl_2$, 3 g/L; NaCl, 1.15 g/L; Na_2HPO_4, 1 g/L; ascorbic acid, 1 g/L; uric acid, 1 g/L; glutathione, pH 7.5 with 0.3/0.6g/L; KOH [13]. Negative control subgingival dental plaque samples were collected from healthy gingival sites with another curette of Gracey sterile and places in the same medium. Each sample collected was suspended in 1 ml transport medium. After homogenization, a 250-µL aliquot was shaken with 0.3 g of acid-washed beads (≤106 mm; Sigma, Saint-Quentin-Fallavier, France) in a FastPrep-24 5G™ instrument (MP Biomedical Europe, Illkirch, France) at a speed of 6.5 m/s (full speed) for 90 s. The supernatant was incubated overnight at 56°C with 180 µL lysis buffer and 25 µL proteinase K (20 mg/ml) in the Qiagen EZ1 DNA tissue kit (Qiagen, Courtaboeuf, France) and total DNA was then extracted using a kit NucleoSpin® (MACHEREY-NAGEL, Düren, Germany). Extraction DNA realize after adding 200 µL B3 buffer, 10 µL synthetic plasmid and incubated for 60 minutes at 70°C. DNA was then washed with 200 µL BW buffer and 600 µL B5 buffer (MACHEREY-NAGEL). After centrifugation for 1600g, the DNA pellet was eluted in 100 µL of EB buffer (heated to 70°C) (MACHEREY-NAGEL). As for PCR amplifications, negative controls consisting of sterile DNA-free water were introduced in all the manipulations. We have performed an amplification of the external synthetic plasmid to check for inhibitor absence in PCR. Methanogen DNA was tentatively detected using PCR-sequencing of the methyl-coenzyme M redutase (*mcr*A) gene (primers mcrALuF 5'-GGTGGTGTMGGATTCACACARTAYGC WACAGC-3'and mcrALuR 5'-TTCATTGCRTAGTTWGGRTAGTT-3 ') and the 16S rRNA gene (primers: 16SrDNA SDArch0333aS15-F 5'-TCCAGGCCCTACGGG-3' and 16SrDNA

SDArch0958aA19-R 5'-YCCGGCGTTGAMTCCAATT-3') as previously described [14]. The PCR program was 94°C for 30s, 58°C for 45s and 72°C for 30s followed by 35 cycles of 10 min at 72°C for amplifying the16S rRNA gene; and 40 cycles at 95°C for 30s, 55°C for 30s and 72°C for 30s followed by extension of 10 min at 72°C for amplifying the *mcr*A gene. PCR product sequences were analyzed with the ChromasPro program, version 1.7: sequence similarity values were determined by BLAST program in the online analysis platform from NCBI (blast.ncbi.nlm.nih.gov). Specific quantitative amplification of the *M. oralis* strain JMR01 [15]. DNA was achieved using a specific real-time PCR assay targeting *M. oralis* heat-shock protein *cnp*60 gene as previously described [12]. Briefly, *M. oralis cnp*602P probe (6-carboxyfluorescein [FAM]-5′AGCAGTGCACCTGCTGATATGGAAGG-3′) (Eurogentec, Seraing, Belgium), *M. oralis-cnp*602F primer (5′-GCTGGTGTAATCGAACCTAAACG-3′) and *M. oralis-cnp*602R primer (5′-CACCCATACCCGGATCCATA-3′) (Eurogentec, Seraing, Belgium) were incorporated into a PCR program comprising of 95°C for 5 min, followed by 40 cycles of 95°C for 15s, 60°C for 35s and 45°C for 30s. The real-time PCR was regarded as positive for any cycle threshold (Ct) value ≤ 40. A calibration curve was established by measuring the Ct values for serial ten-fold dilutions of *M. oralis* (10E+01 to 10E+09). Specific quantitative amplification of the "*M. massiliense"* DNA targeted the "*M. massiliense" mcr*A gene. Primer sequences were determined by using the software primer3 after alignment of the *mcr*A gene sequence from "*M. massiliense"*, *M. oralis*, *M. smithii, M. arboriphilus*, *Methanobrevibacter ruminantium*, *Methanobrevibacter wolinii, Methanobrevibacter cuticularis, Methanobrevibacter filiformis, Methanobrevibacter millerae* and *Methanobrevibacter olleyae*. This yielded a "*M. massiliense" mcr*A probe (6-carboxyfluorescein [FAM] 5′- TGG-CTG-TTC-CAC-TGC-ATT-CGCT-3′) and the primer pair "*M. massiliense"-mcrA* F (5′- ACTCACTTTGGCGGATCTCA-3′**)** and **"***M. massiliense"-mcrA* R (5′- GTACATGGACAAGTACCATGC-3′). The PCR program was 95°C for 5 min, followed by 40 cycles of 95°C for 15s, 60°C for 35 s and 45°C for 30 s. A calibration curve was established by measuring the Ct values of ten-fold serial dilutions of "*M. massiliense"* (10E+01 to 10E+05). Furthermore, real-time PCR targeting the human actin gene was used as an internal calibrator using primer pair Actin F: 5'CATGCCATCCTGCGTCTGGA3' and ActinR: 5'CCGTGGCCATCTCTTGCTCG3' (Eurogentec) and the Actin P6FAM-probe: 5'CGGGAAATCGTGCGTGACATTAAG3' (Eurogentec). The PCR program was 50°C for 2 min, 95°C for 3 min, followed by 45 cycles of 95°C for 15 s, 60°C for 30 s and 45°C for 30 s. A calibration curve was established by measuring the Ct values of serial ten-fold dilutions of HL-60 cells (human leukemia cells) (4.10E+00 to 4.10E+05). Finally, the load ratio of *M. oralis* / human cell and "*M. massiliense"* / human cells

was calculated by dividing the extrapolated number of methanogens in the sample by the extrapolated number of cells using actin quantification as a proxy.

Differences in the prevalence of methanogens in the peri-implantitis sample group and the control sample group were analyses for statistical significance by using the Fisher exact test for discrete values and the Wilcoxon-Mann-Whitney test for ratios; a $P \leq 0.05$ was used to infer significance.

Results

Eight men and twenty women aged between 35 and 86 years diagnosed with peri-implantitis were prospectively included in the study. One control sample and one peri-implantitis sample were collected in each patient, except for patient n°24 in whom three peri-implantitis samples were collected; giving a total of 28 control samples and 30 peri-implantitis samples. A total of 10 individuals had generalized bleeding while 18 had localized bleeding. The pocket depths ranged from 4 to 12 mm. The number of implants affected by peri-implantitis ranged from 1 to 7. In four implants diagnosed with peri-implantitis, pus was observed. The vertical resorption of the supporting bone (% of implant length) ranged from 29% to 100% (100% in only one case) (**Table 1)**. In all PCR experiments, negative controls remained negative and the positive amplification of the external synthetic plasmid control demonstrated the absence of PCR inhibitors.

Using methanogen-specific PCR amplification, 31/58 samples (53.4%) were positive for *M. oralis* with 100% gene sequence similarity with the reference 16S rRNA and *mcr*A gene sequences; comprising 16/30 (53%) peri-implantitis positive samples and 15/28 (53.6%) control samples. Further, 5/58 (8.6%) samples were positive for "*M. massiliense"* with 100% 16S rRNA and *mcr*A gene sequence similarity with reference sequences [9] including 2/30 peri-implantitis samples and 3/28 control samples (**Table 3**). Altogether, methanogens were detected in 36/58 (62%) samples including 17/30 (57%) peri-implantitis samples and 19/28 (67.8%) control samples. Further *M. oralis* and "*M. massiliense"*-targeted PCR amplifications confirmed all the negative and positive results obtained by using methanogen-specific PCR amplifications. There was no statistically significant difference in the prevalence of methanogens in the peri-implantitis and control samples (*M. oralis*, P = 0.61; "*M. massilense"*, P = 0.67; all methanogens, P= 0.45).

Further detection of actin by qPCR extrapolated into 1.15E+01 - 9.29E+05 cells/sample (**Table 2, Table 3**). Actin measurement based deriving the load of host cells which calculated to be $\bar{x}$: 9.10E + 05 in the peri-implantitis samples and $\bar{x}$: 1.68E + 04 in the control samples for *M. oralis* positive sample. Methanogen DNA was detected in 19/28 (67%) control samples with an average number of cells of $\bar{x}$: 1.42E + 04 and in 17/30 (60%) in the peri-implantitis sample with an average number of cells of $\bar{x}$: 8.03E + 05. The mean load ratio of *M. oralis* or "*M. massiliense"* in the peri-implantitis samples was not significantly different than in the control samples (P = 0.48 and P = 0.4, respectively).

Discussion

In this study, we detected the same prevalence of *M. oralis* and "*M. massiliense"'s* DNA in control samples as in the peri-implantitis samples. Both types of samples originated from the same patient, which means that every patient was also his own control. Using this strict methodology and appropriate negative controls which remained negative in all experiments, we observed that the load of both methanogens was not significantly different in the peri-implantitis samples than in the control samples. These results agree with a previous study detecting *M. oralis* in 10 patients with aggressive periodontitis and in 10 periodontally healthy individuals [10]. In a more recent study, *M. oralis* represented 82% of cloned methanogen DNA in patients and 70.1% in healthy individuals [10]. These results however differ from those previously reported using 16S rRNA gene clone library analysis, which failed to recover any methanogen sequences in peri-implantitis samples [3]. We proposed that differences in the methodology partly account for differences in the reported results. Indeed, detecting *M. oralis* in peri-implantitis lesions is not surprising as *M. oralis* is the main methanogen detected in the oral cavity with a prevalence higher than 40% [16, 20]. Here, *M. oralis* was detected in 51% (31/60) of samples. In addition, we also detected "*M. massiliense"* previously reported in the oral cavity with prevalence <20% [8, 9].

However, we propose that the major difference between previous reports and our present report relies on the choice of the control group, i.e. using individuals as their own control as we invented here. Then, our analyses showed an absence of any difference in the prevalence and in the load of methanogens in peri-implantitis samples and control samples collected in the very same person. We argue that these controls are of better value than control samples collected in another individual, as previously reported in studies of peri-implantitis and in studies of periodontitis [10]. The possibility that methanogens from diseased tissues did contaminate healthy tissues cannot be ruled out in our study. This would obviously temperate the conclusions here reported.

Methanogens have been previously detected in another, closely related situation of periodontitis; and that their load was significantly associated with the severity of the periodontal lesions. The results here reported therefore mirror and extend previous data to a yet un-explored situation of peri-implantitis. Indeed, the microbial flora recovered from peri-implantitis lesions differs from that of periodontitis. The genus *Olsenella, Sphingomonas, Peptostreptococcus* and unclassified *Neisseriaceae* are more abundant in peri-implantites whereas the genus *Desulfmicrobium* is lower. The methanogens, although unable to detect species, are more

important in peri-implantitis than periodontitis but without significant difference [17]. In these situations, it has been postulated that the particular metabolism of methanogens, which reduces CO_2 with H_2 as the electron donor for methanogenesis, favors the growth of pathogenic bacteria in anaerobic gingival pockets [10].

Here, we observed that the methanogen load but not the sole detection of methanogens, was not significantly associated with peri-implantitis. This observation differs from that drawn from a previous comparative study of peri-implantitis sites, healthy implant sites and healthy sites [8]. In latter study, authors found a significant difference in the prevalence of methanogens in peri-implantitis sites (48%) and in healthy implants (16%) and natural teeth (8%). Nevertheless, we argue that detecting the presence of methanogens alone is not sufficient to diagnose peri-implantitis, which is probably due to their universal presence in the oral cavity, producing an unavoidable contamination of samples. Only a strict quantification of those methanogens would allow us to overcome this issue. Indeed, we previously made exactly the same observation as for periodontitis [9].

Further studies are warranted to ensure that the observations here reported are not restricted to our center. Indeed, other populations with other geographical and nutritional backgrounds may exhibit different repertoires of methanogens. Cumulative studies will refine our knowledge of the potential role of methanogen in mixed infection with anaerobes in the pathology of peri-implantitis, guiding therapeutic proposals.

Table and figure references

Table 1: clinical data collected in patients with peri-implantitis.

Patient	Sample	Sex	BOP (Bleeding on probing)	PD (Probing depth in mm)	Surfaces with presence of plaque (%)	Presence of pus	Number of implants affected by peri-implantitis	Presence of mobility	Vertical resorption of the supporting bone (% of implant length)
1	P1	f	Generalized	8	50	No	4	No	48
2	P2	f	Generalized	7	100	Yes	2	No	70
3	P3	f	Generalized	7	25	No	1	No	51
4	P4	m	Localized	4	100	No	2	No	37
5	P5	m	Localized	6	100	No	7	No	46
6	P6	f	Localized	4	50	No	2	Yes	29
7	P7	f	Generalized	7	50	No	3	Yes	69
8	P8	f	Localized	8	25	Yes	1	No	51
9	P9	f	Localized	5	25	No	2	No	64
10	P10	m	Localized	4	25	No	1	No	46
11	P11	f	Localized	7	100	Yes	2	No	71
12	P12	f	Localized	12	25	No	1	Yes	100
13	P13	f	Localized	10	50	No	5	No	60
14	P14	f	Generalized	9	100	No	2	Yes	74
15	P15	f	Localized	6	100	No	1	No	52
16	P16	m	Generalized	5	100	Yes	6	No	35
17	P17	f	Localized	7	25	Yes	7	No	67
18	P18	f	Localized	8	100	Yes	1	No	60
19	P19	m	Generalized	7	100	Yes	4	No	57
20	P20	f	Localized	10	100	Yes	2	No	81
21	P21	f	Localized	6	50	No	1	No	50
22	P22	m	Localized	6	100	Yes	1	No	35
23	P23	f	Localized	9	100	No	2	No	40
24	P24, P25, P26	f	Generalized	10	100	No	4	No	85
25	P27	m	Localized	6	100	No	2	No	36
26	P28	m	Generalized	7	100	Yes	2	No	35
27	P29	f	Generalized	9	50	No	4	No	46
28	P30	f	Localized	6	50	No	2	No	35

Table 2: Ratio *Methanobrevibacter oralis* load / human cells load.

	Peri-implantitis			Healthy control		
Samples	*M.oralis* load	Human cells Load	*M.oralis* /Actin ratio	*M.oralis* load	Human cells Load	*M. oralis* /Actin ratio
1	N/A	2,00E+01		5,74E+02	1,66E+02	3,46E+00
2	6,53E+04	6,10E+01	1,07E+03	1,33E+05	1,96E+01	6,79E+03
3	N/A	2,74E+00		N/A	9,11E+01	
4	N/A	7,31E+02		N/A	8,87E+02	
5	1,52E+08	1,51E+01	1,01E+07	3,92E+02	1,17E+02	3,35E+00
6	4,22E+03	7,07E+01	5,97E+01	N/A	2,56E+01	
7	N/A	2,26E+01		5,04E+04	1,15E+01	4,38E+03
8	N/A	6,31E+01		N/A	2,21E+02	
9	1,86E+04	1,81E+01	1,03E+03	5,98E+05	1,37E+01	4,36E+04
10	2,69E+02	3,80E+01	7,08E+00	1,47E+02	2,89E+01	5,09E+00
11	2,08E+05	2,51E+01	8,29E+03	1,50E+03	2,87E+01	5,23E+01
12	N/A	2,21E+01		7,09E+02	1,51E+01	4,70E+01
13	2,42E+02	3,13E+02	7,73E-01	N/A	7,86E+01	
14	N/A	1,01E+04		N/A	1,62E+01	
15	N/A	1,86E+02		N/A	1,63E+04	
16	N/A	6,19E+04		N/A	2,14E+02	
17	2,27E+09	1,74E+04	1,30E+05	3,83E+03	3,05E+03	1,26E+00
18	1,06E+02	6,89E+05	1,54E-04	N/A	1,80E+04	
19	N/A	5,91E+05		1,19E+03	1,12E+05	1,06E-02
20	1,23E+09	3,61E+02	3,41E+06	6,58E+05	3,31E+05	1,99E+00
21	6,58E+05	6,89E+02	9,55E+02	2,43E+05	7,61E+02	3,19E+02
22	N/A	7,41E+02		N/A	3,05E+03	
23	N/A	4,04E+04		7,15E+06	4,55E+04	1,57E+02
24	N/A	2,04E+05		N/A	3,40E+05	
25	4,48E+05	2,71E+05	1,65E+00	7,99E+04	1,17E+03	
26	N/A	9,49E+05		N/A	6,14E+02	
27	4,40E+05	2,04E+03		6,23E+07	2,91E+02	2,14E+05
28	N/A	2,85E+03		5,74E+02	4,87E+05	1,18E-03
29	6,99E+07	2,38E+05	2,94E+02			
30	1,46E+02	3,37E+04	4,33E-03			

N/A: not available

Table 3: Ratio "*Methanobrevibacter massiliensis"* load / human cells load.

	Peri-implantitis			Healthy control		
Samples	***"M.massiliensis"* Load**	**Human cells load**	***"M.massiliensis"* /Actin ratio**	***"M.massiliensis"* load**	**Human cells Load**	***"M.massiliensis"* /Actin ratio**
4	2,06E+02	7,31E+02	2,82E-01	1,08E+02	8,87E+02	1,22E-01
8	7,02E+03	6,31E+01	1,11E+02	5,08E+03	2,21E+02	2,30E+01
18	N/A	6,89E+05	N/A	3,33E+03	1,80E+04	1,85E-01

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V. CORRÉLATION ENTRE LA DETECTION DE *M. ORALIS* ET LE NIVEAU DE RISQUE PÉRI-IMPLANTAIRE

A partir du modèle d'évaluation décrit plus haut, les péri-implantites et les dents contrôles dans lesquelles *Methabrevibacter oralis* a été détectée, ont été groupées et classées dans les trois catégories de risque: élévé, moyen et faible définies (Tableau 1). La comparaison des ratios *M. oralis* / Actine, n'a pas montré des valeurs de ce ratio plus fortes dans la catégorie des péri-implantites à risque élevé, ou à risque moyen par rapport au rique faible.

Ces données vont dans le sens des résultats obtenus dans l'étude effectuée par mademoiselle Souad Belkacemi dans le cadre de l'URMITE et confirme que la présence de *M. oralis* dans les poches de péri-implantites ne peut pas être véritablement corrélée avec la péri-implantite.

Les pathogènes retrouvés dans les poches trouvent probablement des conditions anaérobies favorables de développement et de multiplication. Le fait de retrouver également ces pathogènes dans les *sulci* des dents contrôles en quantité non significativement différente contribue également à penser que ces pathogènes ne font pas partie de la flore spécifique des péri-implantites mais plutôt de la flore commensale.

	Peri-implantites			Dents contrôles			Evaluation suivant le diagramme
Echantillons	*M.oralis* charge	Cellules Humaines charge	*M. oralis* /Actin ratio	*M.oralis* charge	Cellules Humaines charge	*M. oralis* /Actin ratio	Risque
11	2,08E+05	2,51E+01	8,29E+03	1,50E+03	2,87E+01	5,23E+01	Elevé
13	2,42E+02	3,13E+02	7,73E-01	N/A	7,86E+01		Elevé
2	6,53E+04	6,10E+01	1,07E+03	1,33E+05	1,96E+01	6,79E+03	Elevé
18	1,06E+02	6,89E+05	1,54E-04	N/A	1,80E+04		Elevé
20	1,23E+09	3,61E+02	3,41E+06	6,58E+05	3,31E+05	1,99E+00	Elevé
25	4,48E+05	2,71E+05	1,65E+00	7,99E+04	1,17E+03		Elevé
29	6,99E+07	2,38E+05	2,94E+02				Elevé
27	4,40E+05	2,04E+03	2,16E+02	6,23E+07	2,91E+02	2,14E+05	Moyen
5	1,52E+08	1,51E+01	1,01E+07	3,92E+02	1,17E+02	3,35E+00	Moyen
17	2,27E+09	1,74E+04	1,30E+05	3,83E+03	3,05E+03	1,26E+00	Moyen
9	1,86E+04	1,81E+01	1,03E+03	5,98E+05	1,37E+01	4,36E+04	Moyen
10	2,69E+02	3,80E+01	7,08E+00	1,47E+02	2,89E+01	5,09E+00	Moyen
30	1,46E+02	3,37E+04	4,33E-03				Moyen
6	4,22E+03	7,07E+01	5,97E+01	N/A	2,56E+01		Faible
21	6,58E+05	6,89E+02	9,55E+02	2,43E+05	7,61E+02	3,19E+02	Faible

Tableau 1 : Niveau de risque des péri-implantites et des dents contrôles dans lesquelles *M. oralis* a été détectée. La valeur du ratio n'apparaît pas plus importante en fonction du risque.

VI. DISCUSSION

La première étude décrite a été menée pour élaborer une évaluation des risques de péri-implantite, le modèle proposé est basé sur un modèle efficace déjà publié évaluant le risque parodontal (6). Ce précédent modèle a montré son utilité et a permis d'établir un score pour les parodontites. Le modèle actuel a été testé sur vingt-huit patients présentant une pathologie péri-implantaire et a permis de classer ces patients dans trois catégories, chacune correspondant à un niveau de risque d'évolution des péri-implantites (faible : 7.2%, modéré : 35,7%, et élevé : 57.1%).

Le score obtenu fournit des informations quantitatives au clinicien, tandis que le polygone issu du diagramme fonctionnel ajoute une composante qualitative au diagnostic. L'avantage du modèle proposé est donc double : il constitue un outil d'aide au diagnostic tout en standardisant une prise en charge individuelle.

Après avoir effectué une analyse de la littérature, huit paramètres ont été sélectionnés pour le modèle d'évaluation proposé ; cependant plusieurs critères, notamment d'ordre clinique, n'ont pas été inclus. Parmi eux, on peut citer l'occlusion et la quantité de muqueuse kératinisée. Concernant le facteur occlusal, une surcharge biomécanique au niveau de l'interface os-implant pourrait être la cause, ou du moins contribuer à une perte osseuse marginale selon l'hypothèse de la surcharge occlusale. Bien qu'une certaine tension mécanique à l'interface os-implant soit considérée comme souhaitable, les contraintes au-delà d'un certain seuil peuvent aboutir à une déformation de l'os ainsi qu'une réponse biologique incontrôlée caractérisée par l'induction d'un tissu conjonctif fibreux au niveau de l'interface, voire la perte de l'implant.

Quelques études récentes considèrent qu'une charge/surcharge occlusale pourrait être un facteur de contribution à la péri-implantite (15, 16). Mais la question reste ouverte et c'est la raison de la non-inclusion de ce paramètre. Toutefois et bien que les preuves de l'impact d'une surcharge occlusale sur la péri-implantite manquent, une évaluation de l'occlusion des patients durant les visites de maintenance semble souhaitable (17, 18).

Quant à la présence de gencive kératinisée et son niveau d'épaisseur requis en région péri-implantaire, des revues récentes de la littérature semblent montrer que ce paramètre n'a que peu d'influence dans la mesure où une hygiène orale appropriée est maintenue. En revanche, la muqueuse kératinisée, par son absence, peut conduire à une difficulté d'accès et/ou une sensibilité au brossage nuisant au contrôle de plaque et par voie de conséquence, pouvant mener à des lésions tissulaires importantes. Concernant les cas présentant un défaut de gencive kératinisée à proximité implantaire, l'insuffisance/absence de tissu n'affecte pas nécessairement de manière négative la santé des tissus péri-implantaires. Dans l'éventualité où elle nuirait au contrôle de plaque, l'absence de gencive kératinisée s'avère être un paramètre dont l'impact est indirect. Dans le cadre du modèle proposé, il paraissait plus logique d'inclure une évaluation directe de l'hygiène orale, plutôt qu'un critère clinique l'influençant.

Malgré la non-inclusion de ce paramètre dans le modèle d'évaluation, la question de l'importance de la muqueuse kératinisée au niveau péri-implantaire persiste, et ce facteur doit être au centre de l'attention dans les études transversales futures sur l'incidence des maladies péri-implantaires.

Il est possible de comparer le modèle d'évaluation proposé aux travaux de Froum et Rosen (5) sur une nouvelle classification des péri-implantites. Froum classifie la sévérité de la péri-implantite à partir de trois critères cliniques : le saignement et/ou la suppuration au sondage, la profondeur de poche, et le niveau de résorption osseuse péri-implantaire ; l'objectif étant de proposer une classification standardisée servant de pierre angulaire à la communication entre chercheurs et cliniciens et permettant à terme une calibration des études entre elles pour une meilleure comparabilité des résultats. Cette classification proposée par Froum atteint ses limites dans le sens où elle définit un statut implantaire ponctuel à partir de seulement trois critères, tandis que le modèle proposé permet de définir un risque de progression de la péri-implantite, en se basant sur huit paramètres calibrés entre eux. Là où Froum mesure la sévérité de la pathologie à un instant donné, le modèle d'évaluation proposé évalue sa susceptibilité à évoluer dans le temps.

Ces travaux ne sont en rien contradictoires, et peuvent au contraire être corrélés. La comparaison des résultats de cette étude à ceux de Froum et Rosen permet de mettre en

évidence un lien entre la sévérité d'une péri-implantite et sa susceptibilité à s'aggraver : plus une péri-implantite est sévère, plus son risque de progression est élevé. À l'inverse, plus une péri-implantite est précoce, moins son risque d'évolution est important. Cette corrélation vient confirmer le caractère non-linéaire des pathologies péri-implantaires, à fortiori si des facteurs aggravants viennent s'y ajouter (diabète, tabac, parodontopathies, ciment résiduel, mauvaise hygiène orale) comme le montre l'analyse qualitative permise par le diagramme fonctionnel.

Les résultats de l'étude soulignent un paradoxe : d'une part le faible pourcentage de cas identifiés à risque faible de péri-implantite (7,2%) reflétant la difficulté à diagnostiquer les formes débutantes, souvent asymptomatiques avec peu ou pas de manifestations cliniques et faisant le plus souvent l'objet de découvertes fortuites. D'autre part, les thérapeutiques sont d'autant plus efficaces que la prise en charge des péri-implantites est précoce (19).

Concernant la seconde étude, des prélèvements de plaque provenant de péri-implantite et des prélèvements contrôles ont été respectivement collectés chez les mêmes patients, chaque individu constituait donc son propre témoin. L'analyse de ces échantillons a pu mettre en évidence une prévalence identique en ADN de *M. oralis* et *M. massiliensis*. De plus, la concentration de chacun de ces méthanogènes n'était pas significativement différente entre les prélèvements de péri-implantite et les prélèvements contrôle. Ces résultats diffèrent des autres études dans le sens où les tentatives précédentes pour mettre en évidence la présence de méthanogènes dans des lésions péri-implantaires, en se basant sur la détection du gène ARNr 16S, avaient échoué (20, 21). On peut supposer que cette différence est en partie liée à la méthodologie employée dans cette étude. En effet, la détection de *M. oralis* au sein des lésions péri-implantaires ne devrait pas surprendre, la prévalence dans le milieu buccal de cette espèce étant la plus élevée (supérieure à 40%) (13, 22, 23). Ici, *M. oralis* a été détectée dans 51% des échantillons [31/60]. L'espèce « *M. massiliensis »*, dont la prévalence dans la cavité orale est estimée à moins de 20%, a également pu être mise en évidence (9, 12).
La différence majeure entre les précédentes études et notre rapport est le choix du groupe d'échantillons contrôle, faisant de chaque patient son propre témoin. L'analyse montre une absence de différences significatives au niveau de la prévalence et de la concentration en méthanogènes dans les échantillons de péri-implantite et les échantillons de contrôle

collectés chez un même individu. De plus, en établissant la relation du ratio *M. oralis* / actine avec le niveau de risque des péri-implantites, ce ratio n'a pas été plus important chez les péri-implantites à risque élevé.

La concentration en méthanogènes a été corrélée avec la sévérité des lésions parodontales (22). Les résultats obtenus dans ce rapport viennent étendre les données aux péri-implantites. En effet, la flore microbienne récupérée à partir des lésions péri-implantaires varie de celle observée au sein des parodontites. Les genres *Olsenella, Sphingomonas, Peptostreptococcus* et *Neisseriaceae* (non classifiées) sont plus représentés dans les péri-implantites, à l'inverse le genre *Desulfmicrobium* est moins abondant. Les méthanogènes par contre, sans pouvoir distinguer les espèces, sont retrouvés en quantité plus importante dans les péri-implantites que dans les parodontites, mais sans différence significative (24).

VII. CONCLUSION

De la détection précoce à la maintenance régulière, en passant par la rapidité de mise en place des traitements, la prévention a un rôle essentiel à jouer dans la prise en charge des péri-implantites et dans la diminution de leur survenue (14); il est souhaitable de travailler dans ce sens pour les études ultérieures. Il faut également souligner l'aspect multifactoriel de cette pathologie, d'où l'importance d'une compréhension globale des paramètres influant sur le niveau de risque péri-implantaire. Le but étant à terme de catégoriser chaque patient et d'adapter la surveillance thérapeutique de manière individuelle en fonction du niveau de risque de chaque individu.

Cette étude préliminaire devra être complétée par d'autres travaux afin de mettre à l'essai le modèle d'évaluation proposé sur des cohortes de patients plus importantes.

Pour que ce travail soit contributif à l'ensemble des étudiants, praticiens et chercheurs ; l'outil diagnostique a été mis en place sur internet et est accessible à l'adresse suivante : http://diagnostic-tool.pagesperso-orange.fr/. Sa mise à disposition en ligne s'inscrit dans une logique de dépistage et de prise en charge précoce des péri-implantites.

VIII. ANNEXES

Annexe 1 : FORMULAIRE DE CONSENTEMENT

FORMULAIRE DE CONSENTEMENT

Etude entrant dans le champ d'application de la loi Mattei (article 666-8)

Madame, Monsieur,

Vous allez avoir un prélèvement destiné à faire le diagnostic de votre maladie.
Les examens que nous réalisons sont destinés à améliorer le diagnostic, la prise en charge et la surveillance de votre traitement. Il est possible que nous utilisions, de façon anonyme les échantillons prélevés. Ceci afin de tester d'autres méthodes de diagnostic permettant une prise en charge plus précoce ou d'étudier des éléments permettant de mieux comprendre la pathologie.
Le compte rendu des examens réalisés pourrait être utilisé d'une façon tout à fait anonyme. De plus, il est possible que votre cas (sans que l'on puisse vous reconnaître) soit utilisé pour une publication scientifique qui bénéficiera à la connaissance médicale.
Aucun prélèvement supplémentaire ne sera effectué, nous vous demandons l'autorisation d'utiliser les reliquats d'échantillons pour faire des recherches complémentaires plutôt que de les éliminer.

Madame/Monsieur……
…..
ai bien lu que mon observation pouvait être utilisée pour être publiée et que les prélèvements faits, dans le cadre du suivi de ma pathologie, pouvaient être utilisés anonymement à des fins de recherche.

Fait à Marseille, le

Dans tous les cas votre anonymat sera préservé selon les recommandations de la CNIL.

Etiquette Patient

Annexe 2 : QUESTIONNAIRE PATIENT PERI-IMPLANTITES

Données générales

Date du prélèvement **:** ….. / ….. /……. **Profession :** ……

Date de naissance : ….. / ….. /……. **Fumeur :** Non / Fumeur irrégulier / <10 / 10-19 / 20 / >20 cigarettes/jour

Sexe : Homme ☐ Femme ☐

Antécédents médicaux (maladie systémique) .☐……

Si diabète indiquer la dernière glycémie connue : ……..

Traitement systémique ou local .☐……

Prise récente d'antibiotiques : oui ☐ non ☐

Nombre d'implants concernés par la péri-implantite : …….. Localisation : ……Nombre d'implants total : ……

Date de pose du/des implants : ….. / ….. /…….

Switching platform : oui ☐ non ☐

Prothèse supra-implantaire : vissée ☐ scellée ☐

Si prothèse scellée, débordement de ciment : oui ☐ non ☐

Etat de surface : Lisse ☐ Rugueux ☐

Type de prothèse : Coiffe ou bridge implanto-porté ☐

Prothèse amovible supra-implantaire ☐

Prothèse complète type Branemark ☐

Accessibilité à l'hygiène : oui ☐ non ☐

Antécédent de dévissage : oui ☐ non ☐ / Antécédent de fracture : oui ☐ non ☐

Données cliniques :

	oui	non
Traitement parodontal antérieur à la péri-implantite	☐	☐

Date : ….. / ….. /…….

Présence de **saignement** au sondage 0/1/2/3/4/5/6

localisé ☐ généralisé ☐

Présence de **plaque dentaire** 0/1/2/3/4

Présence de **tartre** (indice de Marthaler) 0/1/2/3

Suppuration et nombre d'implants concernés ☐ ☐ 1 2 3 4 5 +

Présence de **mobilité** et nombre d'implants concernés ☐ ☐ 1 2 3 4 5 +

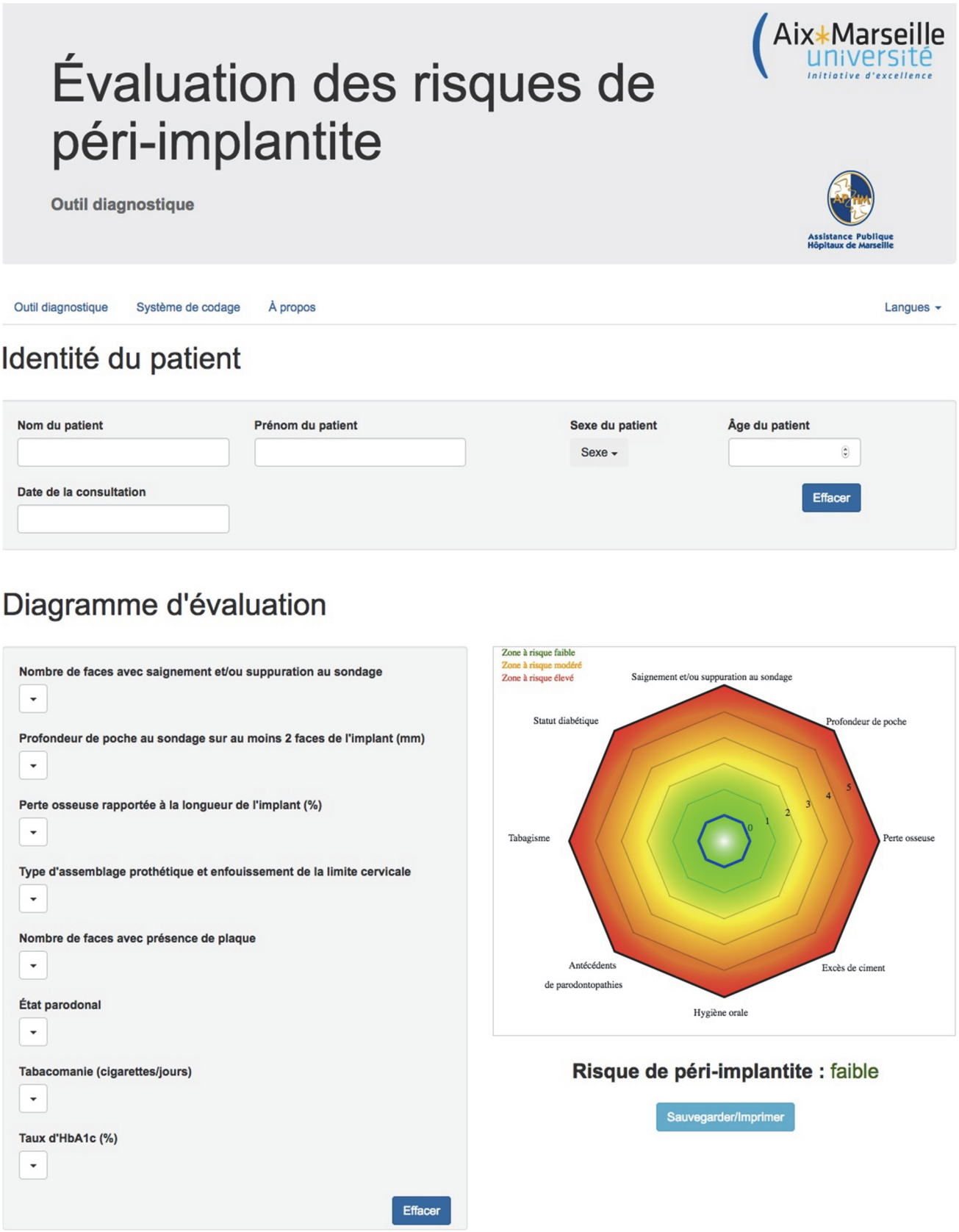
Présence de **tissu kératinisé** en regard du/des implants ☐ ☐

Résorption verticale de l'os de soutien : 1 à 2mm ☐ 2 à 5mm ☐ supérieure à 5mm ☐

Entourer les zones de prélèvement autour du/des implants (**noter la profondeur de poche du site péri-implantaire prélevé)**

11 M - V - D - L	**21** M - V - D - L	**31** M - V - D - L	**41** M - V - D - L
12 M - V - D - L	**22** M - V - D - L	**32** M - V - D - L	**42** M - V - D - L
13 M - V - D - L	**23** M - V - D - L	**33** M - V - D - L	**43** M - V - D - L
14 M - V - D - L	**24** M - V - D - L	**34** M - V - D - L	**44** M - V - D - L
15 M - V - D - L	**25** M - V - D - L	**35** M - V - D - L	**45** M - V - D - L
16 M - V - D - L	**26** M - V - D - L	**36** M - V - D - L	**46** M - V - D - L
17 M - V - D - L	**27** M - V - D - L	**37** M - V - D - L	**47** M - V - D - L
18 M - V - D - L	**28** M - V - D - L	**38** M - V - D - L	**48** M - V - D - L

Annexe 3 : OUTIL DIAGNOSTIQUE EN LIGNE



Système de codage pour le saignement/suppuration au sondage, la profondeur de poche, la perte osseuse et l'hygiène			
Score	**Faces avec saignement et/ou suppuration au sondage**	**Profondeur de poche au sondage sur au moins 2 faces de l'implant (mm)**	**Perte osseuse rapportée à la longueur de l'implant (%)**
0	0	<4	<10
1	1	≥ 4	10-20
2	2	≥ 5	20-30
3	3	≥ 6	30-40
4	4	≥ 7	40-50
5	5-6	≥ 8	>50

Système de codage pour les parodontopathies, le statut glycémique, la tabacomanie et l'hygiène orale				
Score	**État parodontal**	**Taux d'HbA1c (%)**	**Tabacomanie (cigarettes/jour)**	**Nombre de faces avec présence de plaque**
0	Parodonte sain	≥ 6	Non-fumeur	0
1	Parodontite traitée	6,1 - 7	Ancien fumeur	1
2	Parodontite chronique débutante	7,1 - 8	< 10	2
3	Parodontite chronique modérée	8,1 - 9	10 - 19	3
4	Parodontite chronique sévère	9,1 - 10	20	4
5	Parodontite agressive	> 10	> 20	Pas d'accessibilité à l'hygiène

Système de codage pour les excès de ciment en fonction du type d'assemblage et de l'enfouissement de la limite cervicale	
Score	**État parodontal**
0	Prothèse transvissée
1	Prothèse scellée avec limite cervicale supra-gingivale
2	Prothèse scellée avec limite cervicale juxta-gingivale
3	Prothèse scellée avec enfouissement < 1 mm
4	Prothèse scellée avec enfouissement de 1 à 2 mm
5	Prothèse scellée avec enfouissement > 2 mm

Méthode de calcul

Saignement et/ou suppuration au sondage
Sondage clinique léger réalisé sur les 6 faces de l'implant : mésio-vestibulaire, vestibulaire, disto-vestibulaire, mésio-lingual/mésio-palatin, lingual/palatin, disto-lingual/disto-palatin, mesuré après 15 secondes et exprimé en nombre de faces implantaires atteintes (0 à 6).

Profondeur de poche
Sondage clinique léger (0.25 N) à l'aide d'une sonde parodontale graduée réalisé sur au moins deux faces de l'implant et exprimé en millimètres.

Perte osseuse
Évaluée à partir de la radiographie intra-orale rétro-alvéolaire (évaluation de la cratérisation mésiale et distale). Exprimée en pourcentage de résorption osseuse rapporté à la longueur de l'implant.

Excès de ciment
La quantité exacte de ciment résiduel s'avère impossible à mesurer de manière précise, c'est donc la corrélation entre l'enfouissement de la limite cervicale d'une couronne scellée et la quantité de ciment résiduel qui a été exploitée. Les paramètres retenus sont donc : la détermination du moyen d'assemblage pilier implantaire/prothèse (prothèse transvissée ou prothèse scellée) ; dans le cas d'une prothèse scellée, la mesure de la position relative de la limite cervicale par rapport à la gencive marginale en millimètres.

Hygiène orale
Présence ou absence de plaque sur les 4 faces de l'implant : mésiale, distale, vestibulaire et palatine ou linguale et exprimée en nombre de faces implantaires atteintes (0 à 4). Pour certains types de prothèses implanto-portées (prothèses complètes sur pilotis de type Brånemark, bridges implanto-portés), une évaluation complémentaire de l'accessibilité à l'hygiène est proposée (oui/non) : oui si le patient peut se brosser au niveau de la région implantée à l'aide d'une brosse à dent, d'une brossette ou de fil dentaire, non en cas d'accès limité (lié au profil d'émergence notamment) et/ou d'incapacité du patient.

État parodontal
Score calculé en fonction du statut parodontal (sain/traité/pathologique) et du type ou stade de la maladie parodontale.

Tabacomanie
Score mesuré en cigarettes par jour.

Statut glycémique
Score mesuré en pourcentage d'hémoglobine glyquée A1c (HbA1c).

REFERENCE : MAZEL A, BELKACEMI S, ABOUDHARAM G: Peri-implantatis risk factors: a prospective evaluation.

L'outil est consultable à l'adresse suivante :

http://diagnostic-tool.pagespersoorange.fr

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SERMENT MEDICAL

En présence des Maîtres de cette Faculté, de mes chers condisciples, devant l'effigie d'HIPPOCRATE.

Je promets et je jure, d'être fidèle aux lois de l'honneur et de la probité dans l'exercice de la Médecine Dentaire.

Je donnerai mes soins à l'indigent et n'exigerai jamais un salaire au-dessus de mon travail, je ne participerai à aucun partage clandestin d'honoraires.

Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis dans l'intérieur des maisons, mes yeux ne verront pas ce qui s'y passe, ma langue taira les secrets qui me seront confiés et mon état ne servira pas à corrompre les mœurs ni à favoriser le crime.

Je ne permettrai pas que des considérations de religion, de nation, de race, de parti ou de classe sociale viennent s'interposer entre mon devoir et mon patient.

Même sous la menace, je n'admettrai pas de faire usage de mes connaissances médicales contre les lois de l'humanité.

J'informerai mes patients des décisions envisagées, de leurs raisons et de leurs conséquences. Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des connaissances pour forcer les consciences.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

Respectueux et reconnaissant envers mes Maîtres, je rendrai à leurs enfants l'instruction que j'ai reçue de leur père.

Que les hommes m'accordent leur estime si je suis fidèle à mes promesses. Que je sois déshonoré et méprisé de mes confrères si j'y manque.

MAZEL Anthony – Proposition d'un modèle d'évaluation clinique de sévérité des péri-implantites.

Th. : Chir. dent. : Marseille : Aix –Marseille Université : 2017

Rubrique de classement : Implantologie et microbiologie

Résumé :
Une étude a été menée dans le service d'odontologie de l'hôpital de la Timone à Marseille (France) dans le but de créer un outil diagnostique permettant une évaluation du risque d'évolution des péri-implantites en fonction de leur sévérité. Quarante-trois patients, dont 28 diagnostiqués avec une péri-implantite sur au moins un implant et 15 patients contrôles, ont été inclus dans l'étude. Les participants ont rempli un questionnaire, subi un examen clinique approfondi et une radiographie intra-orale rétro-alvéolaire systématique du (des) implant(s) incriminé(s). Les critères suivants ont été observés : le nombre de faces de l'implant présentant un saignement et/ou une suppuration au sondage, la profondeur de poche au sondage sur au moins deux faces de l'implant, la perte osseuse rapportée à la longueur de l'implant évaluée sur les radiographies, le nombre de faces implantaires présentant de la plaque, les paramètres requis pour la détermination des excès de ciment (prothèse vissée ou scellée, enfouissement des prothèses scellées), les statuts parodontal, glycémique et la tabacomanie.
16/28 (57,1%) des cas ont été identifiés à haut risque péri-implantaire contre seulement 2/28 (7,2%) des cas, à faible risque. Les 10/28 (35,7%) restants correspondent aux patients à risque péri-implantaire modéré. Les patients contrôles : 15/15 (100%) présentaient un risque faible.
Par ailleurs, des prélèvements de poches péri-implantaires ont été effectués lors de l'observation clinique pour être analysés par l'URMITE (Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes) Méditerranée Infection. L'objectif était de mettre en évidence l'implication relative des archaea méthanogènes, notamment *Methanobrevibacter oralis* dans la pathogénèse des péri-implantites, à partir des prélèvements sur des implants pathologiques et des dents saines témoins. *M. oralis* et *M. massiliense* ont été retrouvées dans les poches péri-implantaires mais aussi dans les *sulci* des dents saines. Il n'a pas été mis en évidence de corrélation entre la présence et la concentration de ces méthanogènes d'une part, et le niveau de risque péri-implantaire fourni par le modèle proposé.

Mots clés : Péri-implantite, évaluation du risque, modèle d'évaluation, *archaea* méthanogène, *Methanobrevibacter oralis*, « *Methanobrevibacter massiliense* ».

MAZEL Anthony – Proposal of a peri-implantitis risk assessment model

A study was carried out in the odontology department of the Timone hospital in Marseille (France) with the aim of creating a diagnostic tool allowing an evaluation of the risk of peri-implantitis evolution according to the severity. Forty-three patients, 28 of whom were diagnosed with peri-implantitis on at least one implant and 15 control patients, were included in the study approved by the IHU Ethics Committee. Participants completed a questionnaire, underwent a thorough clinical examination and a systematic retro-alveolar intraoral radiography of the incriminated implant(s). The following criteria were observed: the number of faces of the implant showing bleeding and / or pus on probing, the probing depth on at least two faces of the implant, bone loss as a function of the length of the implant, the number of implant faces with dental plaque, the parameters required for the determination of excess cement (screwed or sealed prosthesis, situation in the gums of the sealed prosthesis), the periodontal status, glycemic status and tobacco use. 16/28 (57.1%) of cases were identified with high peri-implantitis risk compared to only 2/28 (7.2%) of cases with low risk. The remaining 10/28 (35.7%) correspond to patients with moderate peri-implantitis risk. Control patients: 15/15 (100%) had a low risk.
In addition, peri-implant pocket samples were taken during the clinical observation to be analyzed by the URMITE (Research Unit on Infectious and Emerging Tropical Diseases) Mediterranean Infection. The aim was to highlight the relative involvement of methanogenic archaea, in particular *Methanobrevibacter oralis,* in pathogenesis of peri-implantitis, using samples from pathological implants and healthy control teeth.
M. oralis and *M. massiliense* were found in the peri-implant pockets but also in the *sulci* of the healthy teeth. No correlation was found between the presence and concentration of these methanogens on the one hand, and the level of peri-implant risk provided by the proposed model.

MeSH : Peri-implantitis, risk assessment, evaluation model, methanogenic archaea, *Methanobrevibacter oralis*, "*Methanobrevibacter massiliense".*

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