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Randomized controlled, clinical blinded study to evaluate the main cause of periodontal disease, the predominant presence of bacteria in percentage and the total bacterial load before and after non-surgical therapy

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Abstract

Goals: Evaluate the root cause of periodontal disease, the predominance of bacteria and the total bacterial load before and after.

Materials and Methods: Microbiological Tests: it consists in taking the crevicular fluid colonized by bacteria and containing epithelial cells of the person being examined, using a sterile paper cone with a diameter of 60/80 in the periodontal or peri-implant pocket for at least 30 seconds (so that it is soaked in liquid crevicular possibly without blood); it is placed inside the test tube; the procedure is repeated to obtain from a minimum of two to a maximum of four paper cones. The test was performed on 420 patients aged 30-60 years, 236 women, 184 men, 4-11mm pockets moderate-severe chronic periodontitis, for a total of 498 samples, in 78 patients a second sampling was carried out after treatment.

Results: The study highlighted that the main cause of periodontal disease are parafunctions (Bruxism, clenching, bad habits, atypical swallowing, mouth breathing), dental misalignment, pre-contacts and incongruous prosthetic products. Therefore, as "direct and triggering" local etiological factors and no longer "indirect and predisposing" as has always been claimed. In the background by bacteria.

Introduction

The periodontal disease: The term periodontal disease (periodontitis) indicates a set of inflammatory pathologies, of an infectious nature, which are characterized at a clinical level by the pathological involvement of all the tissue components of the periodontal organ (gingiva, periodontal ligament, alveolar bone and root cementum) [1-3]. "Direct and triggering" local etiological factors: Bacterial (bacterial plaque, tartar, "alba" ("dawn") material, food residues.

"Indirect and aggravating local" factors: Functional (occlusal trauma, parafunctions, bad habits, oral respiration, atypical swallowing, hypofunction) (Figures 1 -4).

"Indirect and predisposing" factors: Mechanical (wrong oral hygiene, presence of food). Anatomical (dental malposition, dental shape, shape of periodontal tissues).

"Indirect, predisposing and aggravating": Iatrogenic (overflowing fillings, incorrect prosthetic margins, oversized prosthetic crowns and orthodontic devices) [4,5].

The initiation and progression of periodontal disease are commonly attributed to pathogenic bacteria of the oral microbiota, mainly part of the red/orange complexes:

Aggregatibacter actinomycetemcomitans

Porphyromonas gingivalis

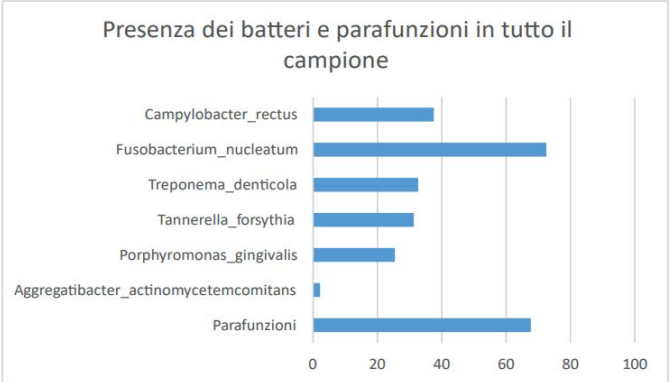

Figure 1. Presence of bacteria and parafunctions in the whole sample before treatment

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Key words: *Periodontal disease, Inflammation, Plaque, Tartar, Bacteria, Microbiological Analysis, Parafunctions*

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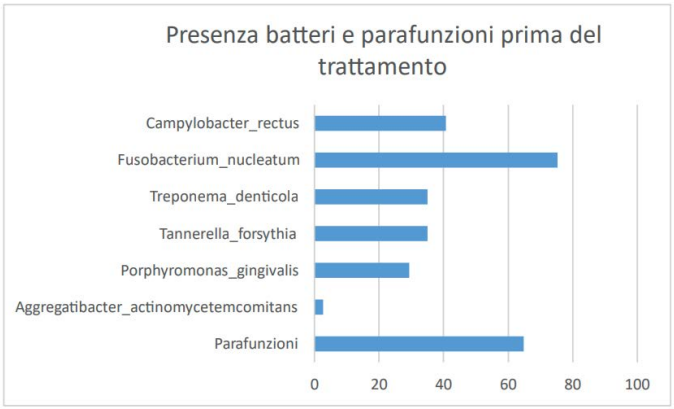


Figure 2. Presence of bacteria and parafunctions before treatment

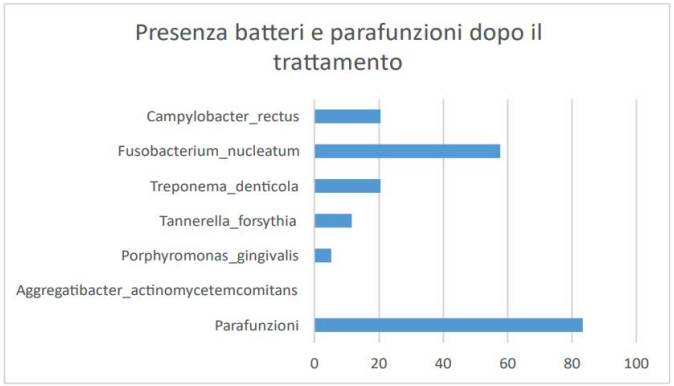


Figure 3. Presence of bacteria and parafunctions after treatment

Tannerella forsythia

Treponema denticola

Fusobacterium nucleatum

Campylobacter rectus

Other factors including bad habits, anatomical and latrogenic, genetic and hereditary factors.

The aim of this study is to evaluate the main cause of periodontal disease, the predominant presence of bacteria in percentage and the total bacterial load before and after non-surgical therapy [6,7].

Materials and Methods

Microbiological Tests: it consists in taking the crevicular fluid colonized by bacteria and containing epithelial cells of the person being examined, using a sterile paper cone with a diameter of 60/80 in the periodontal or peri-implant pocket for at least 30 seconds (so that it is soaked in liquid crevicular possibly without blood); it is placed inside the test tube; the procedure is repeated to obtain from a minimum of two to a maximum of four paper cones. The test was performed on 420 patients, aged 30-60 years, 236 women, 184 men, 4-11mm pockets moderate-severe chronic periodontitis, for a total of 498 samples, in 78 patients a second sampling was carried out after treatment [6-9].

The patients had received no antibiotics or periodontal treatment during the past 6 months, no systemic disease. It follows the methodology, but differs primarily in the choice of patients, with specific characteristics (age, smokers 10/20 cigarettes a day, moderate- severe periodontitis, 4/11mm periodontal pockets) more responsive to ordinary people. Plaque index (IP), bleeding index (BoP), pocket depth (PPD), mobility (M), recession (GAC) was evaluated. All patients were provided with information about the study, having signed the informed consent [10-14].

Clinical Procedures: Microbiological testing was used for the study. The mesial area of the upper right first molar (1.6) and the mesial area of the lower left first molar (3.6) were selected. A group of 78 patients underwent a second sampling after treatment. (Scaling and root planning) (Figures 5-7).

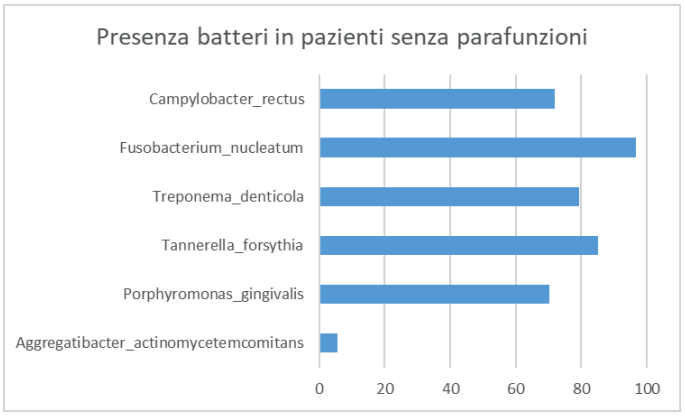


Figure 4. Presence of bacteria without parafunctions

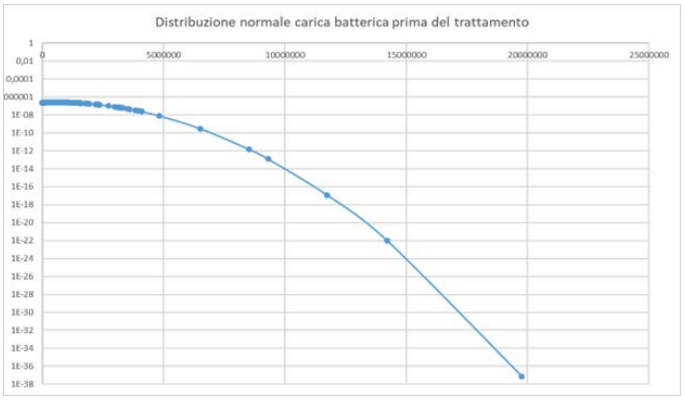


Figure 5. Total bacterial load before treatment

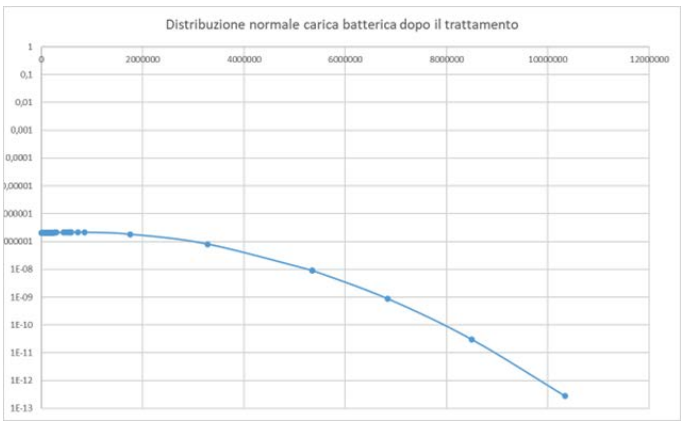


Figure 6. Total bacterial load after treatment

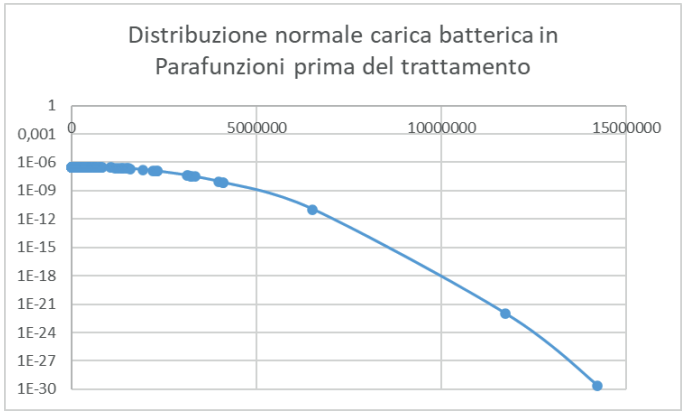
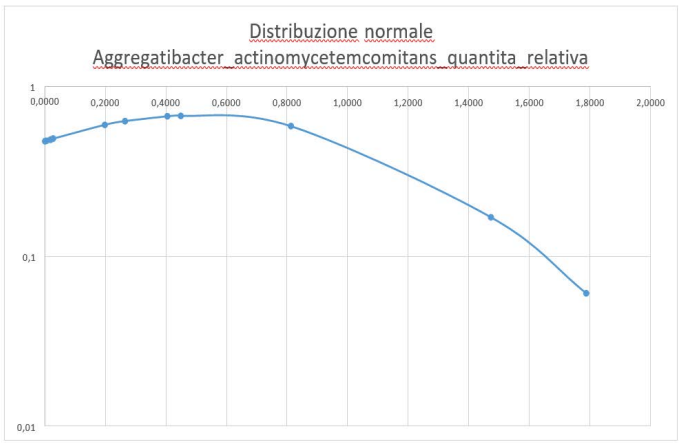
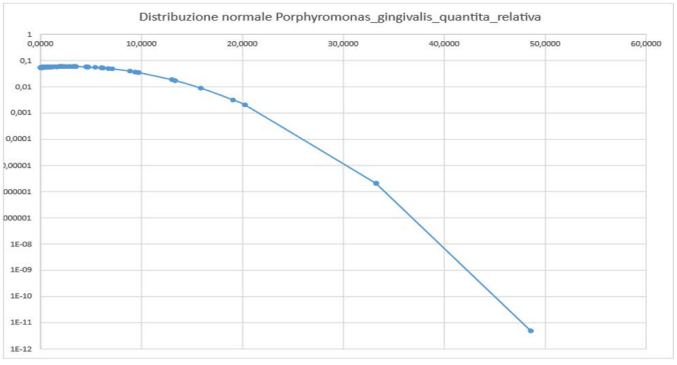


Figure 7. Normal bacterial load distribution in parafunction before treatment



Graph 1



Graph 2

Microbiological Analysis Results

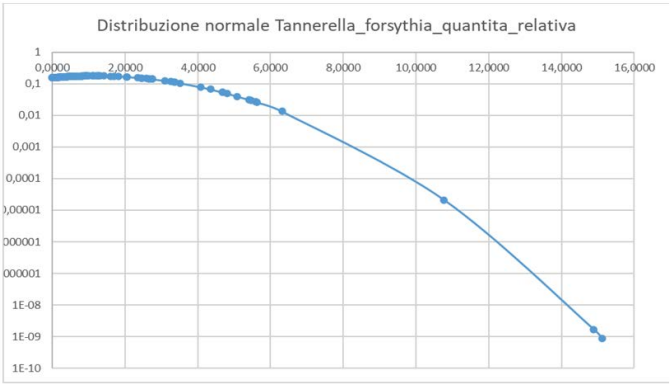
The following sample was analyzed: 498 withdrawals for a total of 420 patients.

In 78 patients a second sampling was carried out after the treatment, for a consideration of 15,7% of the total samples and 18,6% of the patients.

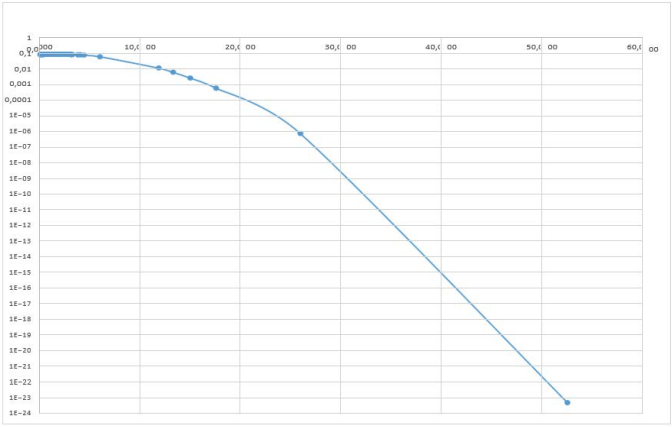
The bacterium *Aggregatibacter actinomycetemcomitans* is present in 2.2% of the samplings (11 cases out of 420) with values ranging from 0,0006% to 1,7875% of the total bacterial load, with an average value of 0,4995% before treatment (Graph 1).

The bacterium *Porphyromonas gingivalis* is present in 25,5% of the samplings (127 cases out of 420) with values ranging from 0,0003% to 48,5433%, with an average value of 2,7917 on the total bacterial load. In the case of patients in which the measurement was made before treatment, the average percentage value is 2,8689, the average value after treatment drops to 0,4178% (Graph 2).

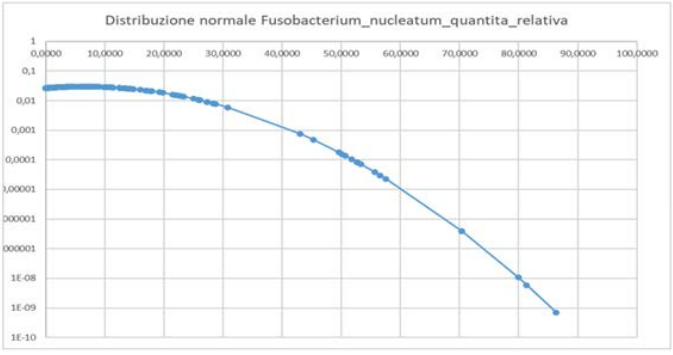
The *Tannerella forsythia* bacterium is present in 31,3% of the samplings (156 cases out of 420) with values ranging from 0,0004% to 15,1197% with an average percentage value of 1.1961 on the total bacterial load. In the case of patients in which the measurement was made before treatment, the average percentage value is 1,2331, the average value after treatment is 0,5912% (Graph 3).



Graph 3



Graph 4



Graph 5

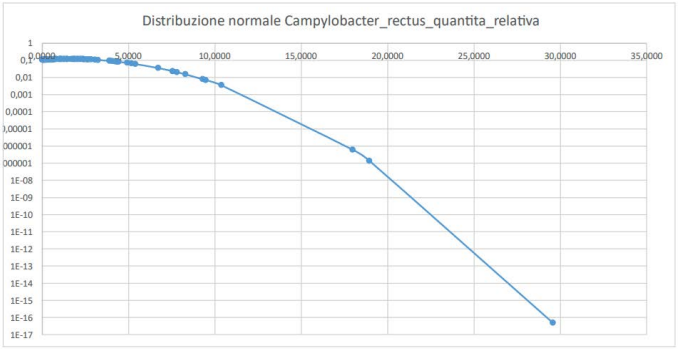
The *Treponema denticola* bacterium is present in 32,7% of the samplings (163 cases out of 420) with values ranging from 0,0010% to 52,4201%, with an average percentage value of 1,5862 on the total bacterial load. In the case of patients in which the measurement was made before treatment, the average percentage value is 1,6668, the average value after treatment is 0,846% (Graph 4).

The *Fusobacterium nucleatum* bacterium is present in 72,5% of the samplings (361 cases out of 420) with values ranging from 0,0035% to 86,3505%, with an average value of 6,4628% of the total bacterial load. In the case of patients in which the measurement was made before treatment, the average percentage value is 7,0148, the average value after treatment is 2,5865 (Graph 5).

The *Campylobacter rectus* bacterium is present in 37,5% of the samplings (187 out of 420 cases) with values ranging from 0,0006% to 29,5476%, with an average value of 1,5387% of the total bacterial load. In the case of patients in which the measurement was made before treatment, the average percentage value is 1,4527, the average value after treatment rises to 2,4589 (Graph 6).

Discussion

The graphs have highlighted that after the treatment (Scaling and root planning) there is a drastic reduction (elimination of some bacteria) of the bacteria and of the total bacterial load. Before treatment the presence of *Aggregatibacter actinomycetemcomitans, Tannerella forsythia,* the percentage is very low and in many cases their absence (Figures 8-12), on the other hand we have a very high percentage of the bacterium *Fusobacterium nucleatum* also present in the intestine and causes



Graph 6

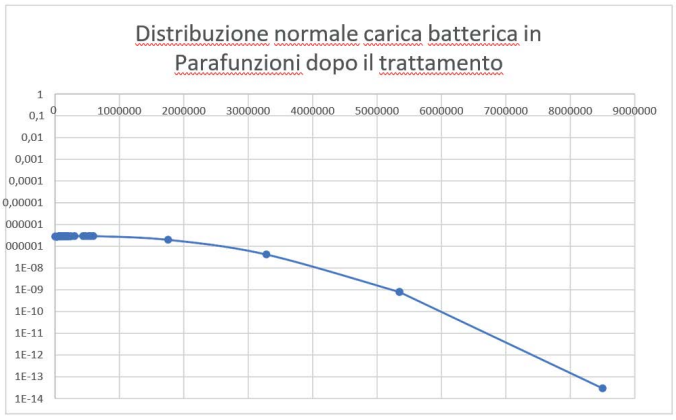


Figure 8. Normal bacterial load distribution in parafunction after treatment

(prima = 1 o dopo = 2)	Codice Paziente	azione (no = 0 o si = 1)	MParafunzioni F	*Aggregatibacter_actinomycetemcomitans*	*Porphyromonas_gingivalis*	*Tannerella_forsythia*	*Treponema_denticola*	*Fusobacterium_nucleatum*	Campylobacte Carica_total r_rectus e		tinomycetemcomitans_quantita_relativa	Porphyromonas_gingivalis_quantita_relativa	Tannerella_forsythia_quantita_relativa
2	Paziente 1	1	Mbruxismo	0	0	0	0	1928	2662	3277397	0,0000	0,0000	0,0000
1	Paziente 2	1	Fprecontatto	0	0	0	1554	1412	0	95789	0,0000	0,0000	0,0000
1	Paziente 3	1	Fserrare	0	0	0	1554	1412	0	95789	0,0000	0,0000	0,0000
0	Paziente 4	1	Fserrare	0	0	0	0	187	0	72376	0,0000	0,0000	0,0000
0	Paziente 5	1	Fprecontatto	0	0	0	2076	35883	1611	519970	0,0000	0,0000	0,0000
0	Paziente 6	1	Fserrare	0	0	0	0	2475	0	174417	0,0000	0,0000	0,0000
0	Paziente 7	1	Fprecontatto	0	0	0	0	0	564	32061	0,0000	0,0000	0,0000
0	Paziente 8	1	Mbruxismo	0	0	0	0	613	580	274734	0,0000	0,0000	0,0000
0	Paziente 9	1	Fbruxismo	0	0	0	0	103	0	118676	0,0000	0,0000	0,0000
0	Paziente 10	1	precontatto	0	0	0	0	0	0	2435	0,0000	0,0000	0,0000
0	Pazi	1	Fserrare	0	0	71	0	28331	0	1434	0,0000	0,0000	0,0495

Figure 9. Bacteria presence in parafunctions

Figure 10. Patient

colorectal cancer (studies have demonstrated a correlation between the bacterium present in the oral cavity and colorectal cancer).

In graph 6, the Parafunctions are 70% (bruxism, clenching, atypical swallowing, precontacts, bad habits, dental misalignment, oral respirator and incongruous prosthetic products), with high bacterial load in some cases without main bacteria in other cases the almost constant presence of the bacterium *Fusobacterium nucleatum*, out of 420 patients 263 are with parafunctions 157 periodontal disease is attributed to bacteria.

Figure 11. Arch

Figure 12. Orthopanoramic

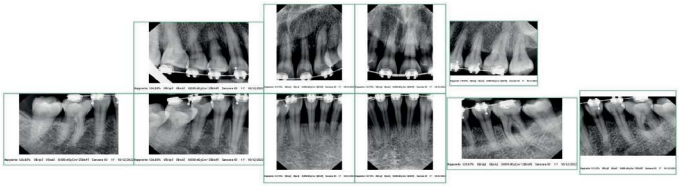


Figure 13. Full intraoral

Of these 263 patients (Chart 15)

N 60 are without bacteria

N 86 are without 5 bacteria

N 112 are without the first 4 bacteria

N 2 only have *Aggregatibacter actinomycetemcomitans*

N 13 have only *Porphyromonas gingivalis*

N 173 have *Fusobacterium nucleatum*

Results

The study highlighted that the main cause of periodontal disease are parafunctions (bruxism, clenching, bad habits, atypical swallowing, mouth breathing), dental misalignment, pre-contacts and incongruous prosthetic products. Therefore as "direct and triggering" local etiological factors and no longer "indirect and predisposing" as has always been claimed. In the background by bacteria. Before treatment the presence of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia*, the percentage is very low and in many cases their absence, on the other hand we have a very high percentage of the bacterium *Fusobacterium nucleatum* (Figures 13-15), also present in the intestine and causing colorectal cancer (studies have shown a correlation of the bacterium present in the oral cavity and colorectal cancer). After the treatment (Scaling and root planning) there is a drastic reduction (elimination of some bacteria) of the bacteria and of the total bacterial load [15-26].

Out of 420 patients 263 are with parafunctions 157 periodontal disease is attributed to bacteria.

Of these 263 patients (Chart 15)

N 60 are without bacteria

N 86 are without 5 bacteria

N 112 are without the first 4 bacteria

N 2 only have *Aggregatibacter actinomycetemcomitans*

N 13 have only *Porphyromonas gingivalis*

N 173 have *Fusobacterium nucleatum*

Out of 78 patients where a second sampling was carried out after treatment, 40% (31 patients) of the patients had *Fusobacterium nucleatum* always present.

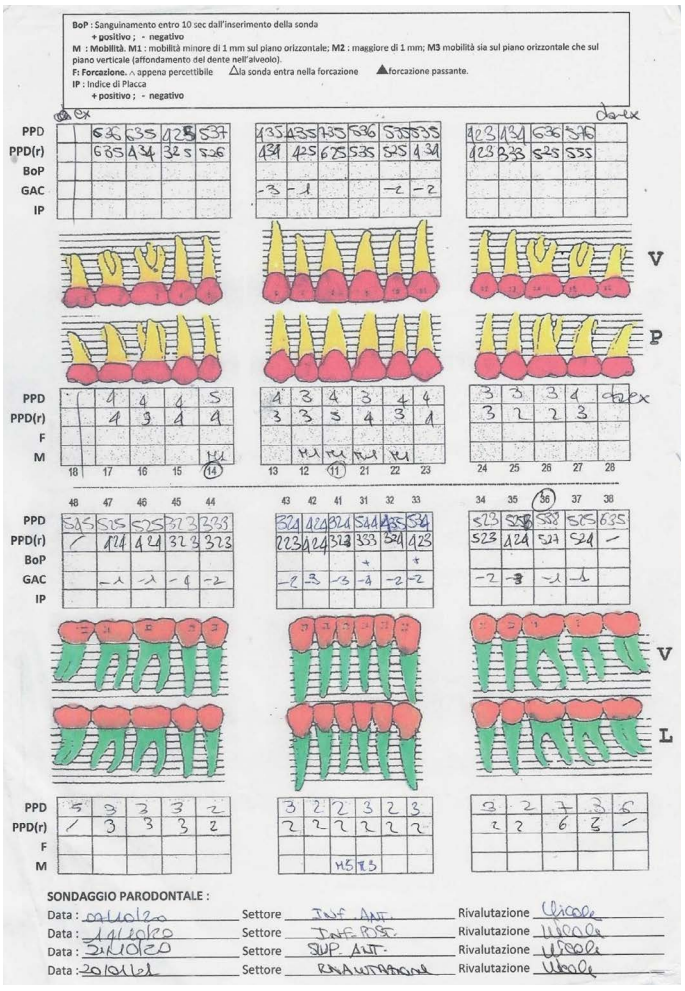
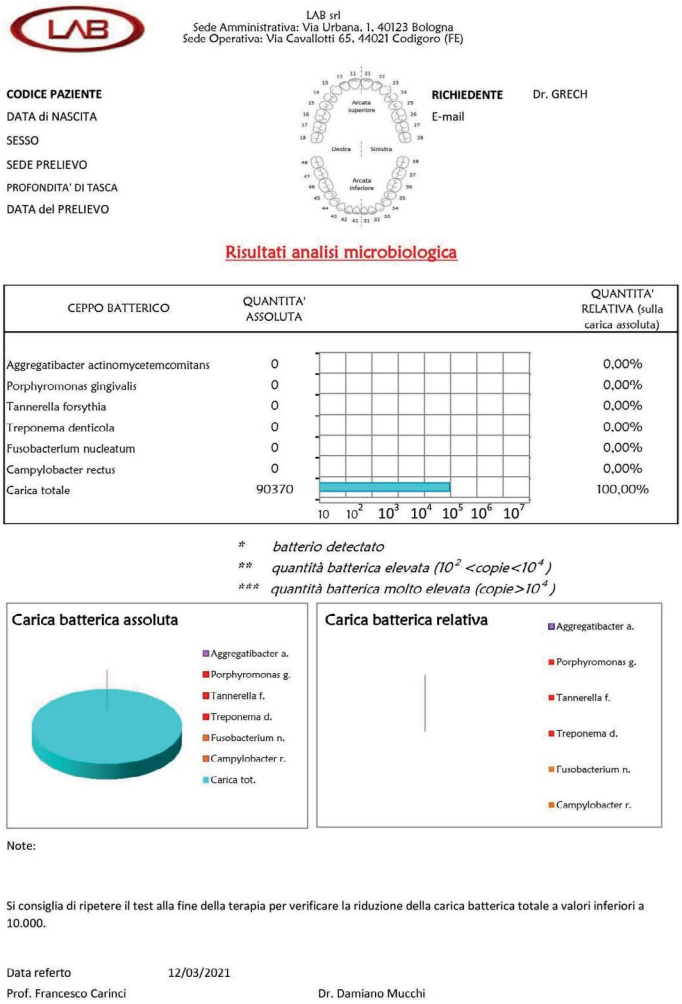


Figure 14. Periodontal chart


LAB srl
Sede Amministrativa: Via Urbana, 1, 40123 Bologna
Sede Operativa: Via Cavallotti 65, 44021 Codigoro (FE)

CODICE PAZIENTE
DATA di NASCITA
SESSO
SEDE PRELIEVO
PROFONDITA' DI TASCA
DATA del PRELIEVO

RICHIEDENTE Dr. GRECH
E-mail

Risultati analisi microbiologica

CEPPO BATTERICO	QUANTITA' ASSOLUTA	QUANTITA' RELATIVA (sulla carica assoluta)
Aggregatibacter actinomycetemcomitans	0	0,00%
Porphyromonas gingivalis	0	0,00%
Tannerella forsythia	0	0,00%
Treponema denticola	0	0,00%
Fusobacterium nucleatum	0	0,00%
Campylobacter rectus	0	0,00%
Carica totale	90370	100,00%

10 10^2 10^3 10^4 10^5 10^6 10^7

* batterio detectato
** quantità batterica elevata (10^2 <copie<10^4)
*** quantità batterica molto elevata (copie>10^4)

Carica batterica assoluta
Aggregatibacter a.
Porphyromonas g.
Tannerella f.
Treponema d.
Fusobacterium n.
Campylobacter r.
Carica tot.

Carica batterica relativa
Aggregatibacter a.
Porphyromonas g.
Tannerella f.
Treponema d.
Fusobacterium n.
Campylobacter r.

Note:

Si consiglia di ripetere il test alla fine della terapia per verificare la riduzione della carica batterica totale a valori inferiori a 10.000.

Data referto 12/03/2021
Prof. Francesco Carinci Dr. Damiano Mucchi

Figure 15. Microbiological test

Acknowledgement

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