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Effects of Smoking on Macrophage Polarization in Peri-Implantitis Lesions

Ettore Amerio[1,2] | Francesco Sparano[1] | Agustín Muñoz-Sanz[3] | Cristina Valles[1] | Jose Nart[1] | Alberto Monje[1,4,5]

[1]Department of Periodontology, Universitat Internacional de Catalunya, Barcelona, Spain | [2]Department of Biomedical, Surgical and Dental Sciences, Unit of Periodontology, University of Milan; Foundation IRCCS Ca' Granda Policlinic, Milan, Italy | [3]Enfermedades Infecciosas. Departamento de Ciencias Biomédicas, Universidad de Extremadura, Badajoz, Spain | [4]Department of Periodontology and Oral Medicine, University of Michigan, Ann Arbor, Michigan, USA | [5]Department of Periodontology, University of Bern, Bern, Switzerland

Correspondence: Alberto Monje (amonjec@umich.edu)

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ABSTRACT

Objectives: The study aimed to investigate the relationship between cigarette smoking and macrophage polarization in peri-implantitis (PI) lesions. Additionally, it sought to characterize clinical, radiological, microbiological, and immunological features of PI in smokers and non-smokers.

Materials and Methods: A cross-sectional study included 40 patients (20 smokers, ≥10 cigarettes/day, and 20 non-smokers) requiring surgical treatment for PI. Samples of peri-implant crevicular fluid (PICF) and granulation tissue were collected during surgery for immunofluorescence and cytokine analyses. Smoking exposure was assessed through cotinine levels. Macrophage polarization (M1/M2) was determined using immunofluorescence. Clinical, radiological, and microbiological parameters were also evaluated.

Results: Smokers showed a significantly higher proportion of M1 macrophages (70.23%) compared to non-smokers (25.09%, $p < 0.005$). This pro-inflammatory shift correlated positively with cotinine levels ($\rho = 0.694$; $p < 0.005$) and pack-years ($\rho = 0.81$; $p < 0.005$). No significant differences in M2 macrophage counts, cytokine concentrations, or microbiota diversity were observed between the groups. However, smokers exhibited more severe PI lesions ($p = 0.04$).

Conclusions: Smoking is associated with a pro-inflammatory shift at the cellular level due to an increase in M1 macrophage polarization in PI lesions, suggesting a pro-inflammatory response that may exacerbate tissue destruction and hinder treatment outcomes. These findings highlight the need for incorporating smoking cessation into comprehensive peri-implant care strategies to improve disease management and implant prognosis.

1 | Introduction

The use of dental implants has seen exponential growth since their development, revolutionizing the treatment of edentulism and tooth loss. However, one of the primary issues that has emerged is peri-implantitis (PI), a pathological condition affecting tissues surrounding dental implants, characterized by inflammation in the peri-implant connective tissue and progressive loss of supporting bone. PI shows increasing prevalence over time post-implantation, affecting on average 18% of implants and between 12% of patients (Rakic et al. 2018). The last World Workshop 2017 pointed out that a history of periodontitis, scarce oral hygiene,

Amerio Ettore and Sparano Francesco should be considered joint first author.

and lack of maintenance are the major risk factors for PI (Schwarz et al. 2018). However, emerging evidence suggests that smoking can also act as an important risk factor to develop peri-implant diseases (Reis et al. 2023; Martinez-Amargant et al. 2023), but the underlying mechanism is not well understood yet.

A comparison of PI samples with periodontitis samples revealed that the area of inflammatory infiltration was more than twice as large in PI samples, with a significantly higher presence of macrophages and plasma cells overall (Carcuac and Berglundh 2014). In periapical tissues affected by periodontitis, a "self-limiting" process occurs upon ligature removal, where a connective tissue capsule separates the inflamed connective tissue from the bone. In contrast, in peri-implant tissues, the inflamed connective tissue extends up to the bone crest (Berglundh et al. 2011). Hence, PI has been associated with the dysregulated immune response of the peri-implant mucosa, with a crucial role played by macrophages. These are essential immune cells that respond to environmental signals and modulate various aspects of inflammation and tissue repair (Carcuac and Berglundh 2014).

Macrophages, which were initially recognized for their phagocytic abilities, play crucial roles in balancing host–microbe interactions, presenting antigens, activating immune defenses, and defending against bacterial infections. These immune cells are known for their ability to adapt to different functions in response to environmental signals. This adaptability is often described as "polarization," which can be mainly categorized into two states following the type-1/type-2 helper cell polarization concept. M1 or classically activated macrophages are typically associated with inflammatory responses and defense against pathogens, whereas M2 or alternatively activated macrophages are involved in inflammation resolution and tissue repair promotion (Yu et al. 2016).

The polarization of macrophages results in distinct functional profiles: M1 macrophages produce pro-inflammatory cytokines, such as TNF-γ, IL-1, IL-6, and IL-12, along with reactive nitrogen and oxygen intermediates when primed by IFN-γ. These responses promote Th1 immune responses with potent bactericidal and antitumor activities. On the other hand, M2 macrophages, primed by IL-4 or IL-13, express markers like arginase 1 (Arg1), CD206, and anti-inflammatory cytokine IL-10, which dampen inflammation to maintain tissue homeostasis. M2 macrophages contribute to parasite control, tissue remodeling, tumor progression, and immunomodulation (Martinez et al. 2008).

Into the bargain, macrophage polarization has been suggested to play an essential role in the pathogenesis of many chronic diseases, including cancer, diabetes, atherosclerosis, and periodontitis (Almubarak et al. 2020; Fretwurst et al. 2020; Garaicoa-Pazmino et al. 2019; Miyajima et al. 2014; Parisi et al. 2018; Stoger et al. 2012; Yu et al. 2016; Zhou et al. 2019).

In a recent study, advanced cases of PI, characterized by severe radiographic marginal bone loss (> 50% of implant length) and deeper probing depths, showed notably higher levels of M1 macrophages. These findings underscore a significant association between increased M1 macrophage expression and the severity of PI (Galarraga-Vinueza et al. 2020). However, research on macrophage polarization in PI is limited; furthermore, the role of potential associated factors, such as smoking and microbiological profile is not clear.

Hence, the primary objective of this study was to examine the relationship between smoking habits and M1/M2 macrophage polarization status. Additionally, the study aimed to explore possible correlations between clinical, radiological, microbiological, and immunological variables.

2 | Materials and Methods

2.1 | Study Design and Population

The research protocol was written following the EQUATOR guidelines (STROBE checklist) and approved by the ethical committee (PER-ECL-2021-05) (E.A., A.M., J.N., C.V. A.M.S.). In this cross-sectional study, a total of 20 smokers (≥10 cigarettes per day) and 20 never-smokers, who attended the Department of Periodontology (Universitat Internacional de Catalunya) for surgical treatment of PI were consecutively recruited (F.S., E.A.). The collection of samples began in September 2022 and concluded in March 2024. Patients gave their express consent to use their data and biological samples for research purposes, and the study was performed following the principles outlined in the Declaration of Helsinki (revised, amended, and clarified in 2013). One implant per patient was considered; if more than one implant met the inclusion criteria, one implant was randomly selected.

2.2 | Definitions

PI was defined according to the definition proposed by the 2017 World Workshop (Berglundh et al. 2018). The case definition applied was as follows: the presence of bleeding and/or suppuration on gentle probing (0.25 N), probing pocket depths of ≥6 mm, and bone levels ≥3 mm apical to the most coronal portion of the intraosseous part of the implant based on periapical X-ray. The severity of the PI was based on the percentage of bone loss (slight < 25%, moderate 25%–50%, and advanced > 50%) calculated based on the position of the peri-implant bone with respect to the implant neck and the total implant length (Monje et al. 2019).

2.3 | Inclusion and Exclusion Criteria

Patients who needed surgical treatment for PI were included. The exclusion criteria were: (1) general contraindications for dental and surgical treatments, (2) untreated periodontal disease, (3) pregnant or lactating women, (4) autoimmune or inflammatory diseases, (5) uncontrolled diabetes (HbA1c > 7), (6) corticosteroid therapy, (7) former smokers, (8) light smokers (< 10 cigarettes per day), (9) patients who had taken antibiotics in the last 3 months, (10) no previous surgical or non-surgical treatment of PI of the implant under evaluation.

2.4 | Clinical and Radiological Examination

After recording the medical history, the examiner collected data regarding age, gender, stage/grade/extent of periodontitis (30), smoking status (number of cigarettes per day/years of

smoking), pack-years, implant position, implant design (tissue/bone level), and function time of the implant. Subsequently, the following implant parameters and indexes were assessed at 6 sites per implant by one experienced and calibrated investigator (F.S.): Bleeding on probing (BOP) (within 30 s); Modified Plaque Index (mPI) (Mombelli et al. 1987); Probing Pocket Depth (PPD); Suppuration (SUP). In addition, the Implant Mucosal Index (IMI) (French et al. 2016) was assessed. One periapical radiograph of the implant concerned was taken by means of a paralleling device, and the marginal bone level (MBL) was assessed by a calibrated examiner using a software program (ImageJ) based on the known length of the implant or the distance between threads (Schneider et al. 2012).

2.5 | Sample Collection and Histological Processing

All patients received pre-operative professional supragingival tooth/implant cleaning and were treated through a standardized surgical protocol. Prior to the intervention, peri-implant crevicular fluid (PICF) was collected by a trained and calibrated examiner (F.S.). The PICF samples were collected during early morning hours (between 9 AM and 10 AM). In summary, participants were instructed to avoid eating, drinking, and/or smoking at least 1 h prior to PICF sample collection. The participants were comfortably seated on a dental chair, and the peri-implant supragingival plaque was gently removed. A saliva ejector was also used during the sampling procedure. Samples were collected as follows:

- Cotinine, Cytokine, and Microbiological Samples: Six sterile endodontic paper points were placed in the peri-implant sulcus for 30 s. Each set of samples was placed in microcentrifuge tubes. Cotinine sample tubes contained 1 mL of phosphate-buffered saline. All samples were frozen at −80°C until further processing.
- Histological samples: Following local anesthesia, buccal and lingual mucoperiosteal flaps were elevated to expose the peri-implant defect. All granulation tissue was carefully and circumferentially harvested from the respective intrabony defect areas using conventional plastic curettes, rinsed with saline, and a paraformaldehyde 4% was used for fixation and stabilization until further processing.

All samples were stored as coded specimens to avoid revealing personal patient-related information. The biological samples were stored at Universidad Internacional de Catalunya and subsequently sent to the laboratory (Servicio de Técnicas Aplicadas a las Biociencias (STAB), Edificio Guadiana Avd. Elvas, s/n 06071 BADAJOZ) for further analysis (A.M.S.).

2.6 | Immunofluorescence

The methodology to identify M1 and M2 macrophages using immunofluorescence techniques was previously described in detail (Fretwurst et al. 2020). Briefly, primary mouse monoclonal antibodies anti-CD68, anti-iNOS, and anti-CD206 were adopted. Subsequently, the iNOS++/CD68++ (M1-like macrophage) and CD206++ (M2-like macrophage)-positive cells were analyzed and quantified using monochromatic images with the NIH ImageJ software. Three masked, calibrated examiners did the analyses. Subsequently, macrophage-related variables, such asthe number of macrophages/μm^2, the number of M1 and M2 macrophages/μm^2, and the M1 proportion (M1/M1 + M2) were calculated.

2.7 | Cytokine and Chemokine Assays

The concentrations of the following cytokines in the PICF sample were assessed using immunoassays formatted on magnetic beads (Bio-Plex Pro Assays, Bio-Rad Laboratories Ltd., UK): tumor necrosis factor (TNFα), interferon-γ (INF-γ), Monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), Interleukin-2 (IL-2).

2.8 | Microbiological Analysis

The analysis of the microbiological samples was performed as previously described by using 16S rRNA pyrosequencing methods (Tsigarida et al. 2015) to obtain the peri-implant biofilm characterization.

2.9 | Assessment of Cotinine Levels

Cotinine levels in the PICF were assessed by a calibrated and experienced investigator, who was masked to the study groups. Concentrations of cotinine were determined using an enzyme-linked immunosorbent assay kit (High Sensitivity Salivary-Cotinine-Quantitative-Enzyme-Immunoassay Kit, Salimetrics, State College, Pennsylvania) as per the manufacturer's guidelines. The minimum concentration that could be measured using this kit is 0.15 ng/mL.

2.10 | Sample Size Calculation

The primary variable was the mean M1 proportion (M1/[M1 + M2]). Assuming a standard deviation of the mean M1 proportion of 0.16 (Galarraga-Vinueza et al. 2020) a change of at least 0.15 between smokers and non-smokers, an alpha error of 5%, a beta error of 20%, and a two-sided test (*Z*-statistics), 18 patients per group (18 implants) were required. To account for potential dropouts due to the possible unreadability of some histological samples, 20 patients per group were recruited.

2.11 | Statistical Analysis

The statistical analysis was conducted by using a commercially available software program (SPSS, 26, Chicago, IL, USA) (E.A.). Descriptive statistics were performed for each variable at the patient level (=implant level). The Shapiro–Wilk normality test was adopted to test the normal distribution of the data in the case of continuous variables, and parametric and non-parametric tests were used accordingly to test mean differences. Chi-squared or Fisher tests were used to test differences for categorical variables.

Correlations were analyzed using either the Pearson correlation coefficient for data with a normal distribution and a linear relationship, or the Spearman correlation coefficient for data that did not meet these criteria.

For the microbiological analysis, the relative abundance of the target species was calculated for each sample, and the Simpson index was used to assess the α-diversity. Differences in relative abundances between smokers and non-smokers were tested using the non-parametric Mann–Whitney *U* test, and the Benjamini-Hochberg method was applied to correct for multiple comparisons.

Generalized linear models were employed, using the M1 proportion as the dependent variable, to conduct multivariate analysis and account for potential confounding factors. A *p*-value of less than 0.05 was used as the threshold to determine the significance of all analyses.

3 | Results

3.1 | Study Population

Overall, 20 smokers and 20 non-smokers, all medically healthy patients, were included in the study. However, five histological samples (3 smokers and 2 non-smokers) could not be analyzed due to the small size of the histological sample and were excluded from data analysis. Hence, 35 patients were finally analyzed. Additionally, three patients in the non-smoker group were excluded from the microbiological analysis due to insufficient bacterial DNA extraction.

Patients were 21 males (60%) and 14 females (40%) with an average age of 63.5 years (SD 10.5). Implants were mostly bone-level implants (85.7%) and were primarily located in the molar– premolar area (77.1%) and the upper jaw (65.7%). The severity of the peri-implant disease was predominantly advanced (51.4%), whereas the rest of the patients presented with moderate to slight PI (moderate: 31.4%, slight: 17.1%) (Table 1).

Smokers smoked an average of 15.1 cigarettes (range: 10–25; SD 5.0) and had a history of smoking ranging from 20 to 50 years (mean: 37, SD 7.0), resulting in an average of 27.9 pack-years (SD 9.9) (Table 1).

3.2 | Smokers Versus Non-Smokers

Non-smokers were significantly older than smokers ($p = 0.01$). Smokers presented a more severe stage ($p = 0.024$) and grade of periodontitis ($p < 0.005$). In addition, most of the implants in the non-smoker group were placed in the maxilla ($p = 0.035$). Notably, smokers presented with more severe PI lesions ($p = 0.04$). No other differences were found between groups in any other demographic, clinical, and radiographical variables (Table 1). The observed mean differences in cotinine levels in PICF between smokers and non-smokers were, as expected, highly significant ($p < 0.005$). However, no significant correlations were found between cotinine levels and the number of cigarettes per day ($\rho = -0.28$, $p = 0.915$), years of smoking ($\rho = -0.105$, $p = 0.688$), or pack-years ($\rho = 0$, $p = 1$) in the smoker group.

3.3 | Macrophage Polarization

The M1 proportion in smokers was 70.23% (SD 9.65%), whereas in non-smokers it was 25,09% (SD 9.96%) and the difference was highly significant ($p < 0.005$; Figures 1 and 2; Table 2). Smokers had a higher number of M1 compared to non-smokers, whereas the difference in the number of M2 was not significant between groups (Figures 1 and 2; Table 2). Likewise, patients displaying more severe stages of periodontitis presented a higher M1 proportion ($p = 0.010$).

No differences were found in terms of M1 proportion considering severity ($p = 0.239$), gender ($p = 0.21$), implant position (anterior vs. posterior) ($p = 0.893$), implant position (maxilla vs. mandible) ($p = 0.208$; Table 3).

Significant correlations between M1 proportion and age ($\rho = 0.377$; $p = 0.026$), cotinine ($\rho = 0.694$; $p < 0.005$), pack-years ($\rho = 0.81$; $p < 0.005$) were found (Table 4).

Generalized linear models revealed that pack-years ($B = 0.13$; 95% CI: 0.10–0.17; $p < 0.005$) was the only significant factor associated with M1 proportion in a multivariate model including Age ($B = 0.002$; 95% CI: −0.003 to 0.007; $p = 0.504$) and Stage of Periodontitis ($B = 0.015$; 95% CI: −0.043 to 0.072; $p = 0.611$).

3.4 | Cotinine and Cytokine Profile

The observed mean differences in cotinine levels in PICF between smokers and non-smokers were, as expected, highly significant ($p < 0.005$). However, no significant correlations were found between cotinine levels and the number of cigarettes per day ($\rho = -0.28$, $p = 0.915$), years of smoking ($\rho = -0.105$, $p = 0.688$), or pack-years ($\rho = 0$, $p = 1$) in the smoker group. No significant differences between cytokine concentrations were found comparing smokers and non-smokers (Table 1). Significant correlations were found between Age and MCP-1 concentration ($\rho = 0.35$; $p = 0.039$); Mean BOP and IL-8 concentration ($\rho = 0.34$; $p = 0.043$); Mean mPI and TNF-α ($\rho = 0.44$; $p = 0.440$) and INFγ ($\rho = 0.442$; $p = 0.008$); MBL max and IL-8 ($\rho = 0.388$; $p = 0.021$); mean PPD and IL-8 ($\rho = 0.570$; $p < 0.005$) and IL-2 ($\rho = -0.460$; $p = 0.005$; Table 5). No significant correlations were found between any cytokine and cotinine, pack-years, function time, IMI, stage of periodontitis, and macrophage-related variables except for a negative correlation between INFγ and the number of macrophages/μm^2 ($\rho = -0.434$; $p = 0.009$; Table 5).

3.5 | Microbiological Results

No differences were found between smokers and non-smokers in terms of richness and α-diversity of the microbiota (Table 1). Still, a positive correlation between the α-diversity (Simpson index) and the cotinine concentration was found ($\rho = 0.462$; $p = 0.008$). No significant correlations were found between α-diversity and macrophage-related variables. The three most abundant species were *Porphyromonas gingivalis, Parvimonas micra*, and *Peptostreptococcus stomatis*. No significant differences were

TABLE 1 | Comparison between smokers and non-smokers in terms of demographic, clinical, and radiological variables, cytokine concentrations, and microbiota richness and diversity.

	Non smokers		Smokers		*p*
	N	**%**	***N***	**%**	
Gender					0.305
Male	9	50.00%	12	70.59%	
Female	9	50.00%	5	29.41%	
Stage periodontitis					
I	0	0.00%	0	0.00%	**0.024**
II	10	55.56%	2	11.76%	
III	2	11.11%	4	23.53%	
IV	6	33.33%	11	64.71%	
Grade of periodontitis					**< 0.005**
A	5	27.78%	0	0.00%	
B	7	38.89%	0	0.00%	
C	6	33.33%	17	100.00%	
Extent of periodontitis					
Localized	2	11.11%	1	5.88%	1
Generalized	16	88.89%	16	94.12%	
Implant position (Ant-Post)					
Posterior	4	22.22%	4	23.53%	1
Anterior	14	77.78%	13	76.47%	
Implant position (Max-Mand)					
Mandible	3	16.67%	9	52.94%	**0.035**
Maxilla	15	83.33%	8	47.06%	
Implant design					
Tissue level	2	11.11%	3	17.65%	0.658
Bone level	16	88.89%	14	82.35%	
Severity PI					
Slight	3	16.67%	3	17.65%	**0.04**
Moderate	9	50.00%	2	11.76%	
Advanced	6	33.33%	12	70.59%	
SUP	6	33.33%	10	58.82%	0.24
	Mean	**SD**	**Mean**	**SD**	
Age	66.53	11.88	59.71	7.56	**0.01**
Cotinine (ng/mL)	0.044	0.098	71 255.96	99 319.95	**< 0.005**
Function_time	11.53	6.2	11.71	6.02	0.757
Mean_PPD	6.63	2.36	7.24	1.73	0.293
Mean_BOP	0.77	0.29	0.92	0.13	0.184
Mean_mPI	1.12	0.69	1.11	0.55	0.961

(Continues)

TABLE 1 | (Continued)

	Non smokers		Smokers		*p*
KM	1.06	1.6	1.76	1.68	0.205
IMI	3.12	0.99	3.59	0.51	0.134
MBL_mesial	5.65	2.84	6.83	2.18	0.053
MBL_distal	5.91	2.76	6.47	1.95	0.303
MBL_max	6.14	2.78	6.92	2.14	0.232
TNF-α (pg/mL)	172.12	244.37	168.07	227.15	0.318
MCP_1 (pg/mL)	60.3018	91.76	35.21	18.37	0.405
INF-γ (pg/mL)	70.6	186.79	47.43	162.77	0.361
IL_8 (pg/mL)	1676.16	4813.82	1537.42	1939.42	0.103
IL_2 (pg/mL)	36.7	95.21	24.97	80.61	0.757
Simpson Index ($N = 17$ S; $N = 15$ NS)	0.56	0.18	0.67	0.22	0.16
Observed diversity	6.82	2.16	6.67	1.29	0.808

Note: Figures in bold indicate statistical significance ($p < 0.05$).
Abbreviations: BOP, Bleeding on Probing; IL, Interleukin; IMI, Implant Mucosal Index; INF-γ, Interferon Gamma; KM, Keratinized Mucosa; MBL, Marginal Bone Loss; MCP_1, Monocyte Chemoattractant Protein 1; PI, Peri-implantitis; PPD, Probing Pocket Depth; SUP, Supuration; SD, Standard Deviation; TNF-α, Tumor Necrosis Factor Alpha.

found in the relative abundance of the target species between smokers and non-smokers (Table 6).

4 | Discussion

4.1 | Principal Findings

The primary objective of this study was to examine the relationship between smoking habits and M1/M2 macrophage polarization status in PI lesions. The findings demonstrated that smokers exhibited a significantly higher proportion of M1 macrophages compared to non-smokers in PI lesions, indicating a pro-inflammatory response. Specifically, the M1 proportion in smokers was 70.23%, whereas in non-smokers, it was 25.09%, highlighting a substantial difference between the two groups. A dose-effect relationship was also present, with the M1 proportion significantly associated with pack-years and cotinine levels. As a higher proportion of M1 macrophages was proved, the present study proposes a potential pathway through which smoking plays a role in the onset and/or exacerbates the progression of peri-implant disease, thereby worsening the prognosis of dental implants in smokers. Moreover, this suggests that smoking may influence key mechanisms involved in the disease process, potentially complicating treatment outcomes aimed at achieving disease control and stability around dental implants.

4.2 | Agreement and Disagreement With Previous Findings

To our knowledge, this is the first study that analyzed the role of smoking in the macrophage polarization around dental implants. Hence, comparison with previous studies is challenging. The relationship between smoking and PI has been a topic of debate over the years, with some studies suggesting a potential association between the two conditions (de Araújo Nobre et al. 2015; Reis et al. 2023; Renvert et al. 2014; Rinke et al. 2011; Roos-Jansåker et al. 2006a, 2006b; Schwarz et al. 2017), while others have found no such relationship (Aguirre-Zorzano et al. 2015; Canullo et al. 2016; Casado et al. 2013; Dalago et al. 2017; Daubert et al. 2015; Dvorak et al. 2011; Koldsland et al. 2010, 2011; Marrone et al. 2013; Máximo et al. 2008; Rokn et al. 2017). The role of potential confounders, particularly a history of periodontitis, along with variations in how PI and smoking status are defined, can partially account for these discrepancies (Schwarz et al. 2018).

Recent studies suggest that macrophage polarization may play a key role in the pathogenesis of peri-implant diseases. A skew towards the M1 macrophage population was previously demonstrated in other studies comparing periodontal health and periodontal disease (Yu et al. 2016; Zhou et al. 2019) and more recent findings suggested that the M1 proportion is even higher in peri-implant disease compared to periodontal disease (Dionigi et al. 2020; Fretwurst et al. 2020). Lately, an association was found between peri-implant disease severity and M1 polarization suggesting a key role of this aggressive macrophage population in disease progression (Galarraga-Vinueza et al. 2020). Previous research has demonstrated that bacterial by-products, such as lipopolysaccharides, and interferon-gamma (IFN-γ) can trigger pro-inflammatory responses in host tissues by activating M1 macrophages, thereby promoting inflammation (Gao et al. 2018; Zhuang et al. 2019). In chronic inflammatory conditions, CD4 T cells can differentiate into Th1 or Th2 subsets, either enhancing or suppressing inflammation and consequently influencing macrophage polarization. The impact of smoking on adaptive immunity remains a topic of debate in the literature; however, a shift toward the Th1 response has been observed around healthy dental implants in smokers (Negri et al. 2016). The present study did not analyze the presence of Th1 or Th2 cells in the peri-implant tissue; however, no differences in Th1 cytokines, such as IL-2, TNF-α,

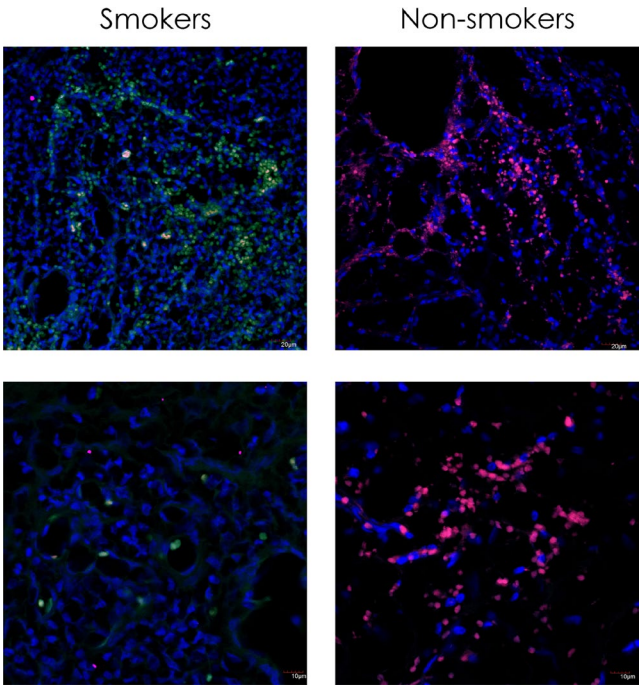

FIGURE 1 | Immunofluorescence analysis showing CD68 (green, FITC), iNOS (orange, Alexa 561), and CD206 (red, PercP). M1 macrophages appear yellow-green due to co-localization of CD68 and iNOS, while M2 macrophages appear orange-red indicating CD206 expression. Representative images demonstrate a higher prevalence of M1 macrophages in smokers.

and IFN-γ were observed in the PICF of smokers and non-smokers. Numerous studies in general medicine have demonstrated that exposure to cigarette smoke influences inflammation, modulates macrophage phenotypes, and alters various macrophage functions, including microbial phagocytosis (Lee et al. 2012; Park et al. 2018; Phaybouth et al. 2006; Shaykhiev et al. 2009; Thomas et al. 1978). In contrast to the findings of the present study, previous evidence showed that cigarette smoke dose-dependently reduces M1 polarization in macrophages (Bazzan et al. 2017; Bianchi et al. 2024; Shaykhiev et al. 2009; Yuan et al. 2014). This decrease in the M1 phenotype suggests a diminished ability to combat initial inflammation and infection. However, some studies demonstrate the opposite effect (Eapen et al. 2017; Karimi et al. 2006). These drastically opposite outcomes underscore an ongoing debate in the field regarding the impact of cigarette smoke on pro-inflammatory and anti-inflammatory responses. One reason that may explain these discrepancies is that most studies focus on the initial stimuli of tobacco on normal cells. This contrasts with disease states where the pathological condition has progressed beyond the initial exposure and may have already developed, worsened, or subsided (Yang and Chen 2018).

In the present sample, smokers exhibited a higher number of M1 macrophages compared to non-smokers, while the number of M2 macrophages remained comparable. This was accompanied by an overall increase in total macrophages. Therefore, it may be speculated that the vast majority of macrophages present in peri-implantitis lesions in smokers are less likely to be tissue-resident and more likely to have originated as circulating

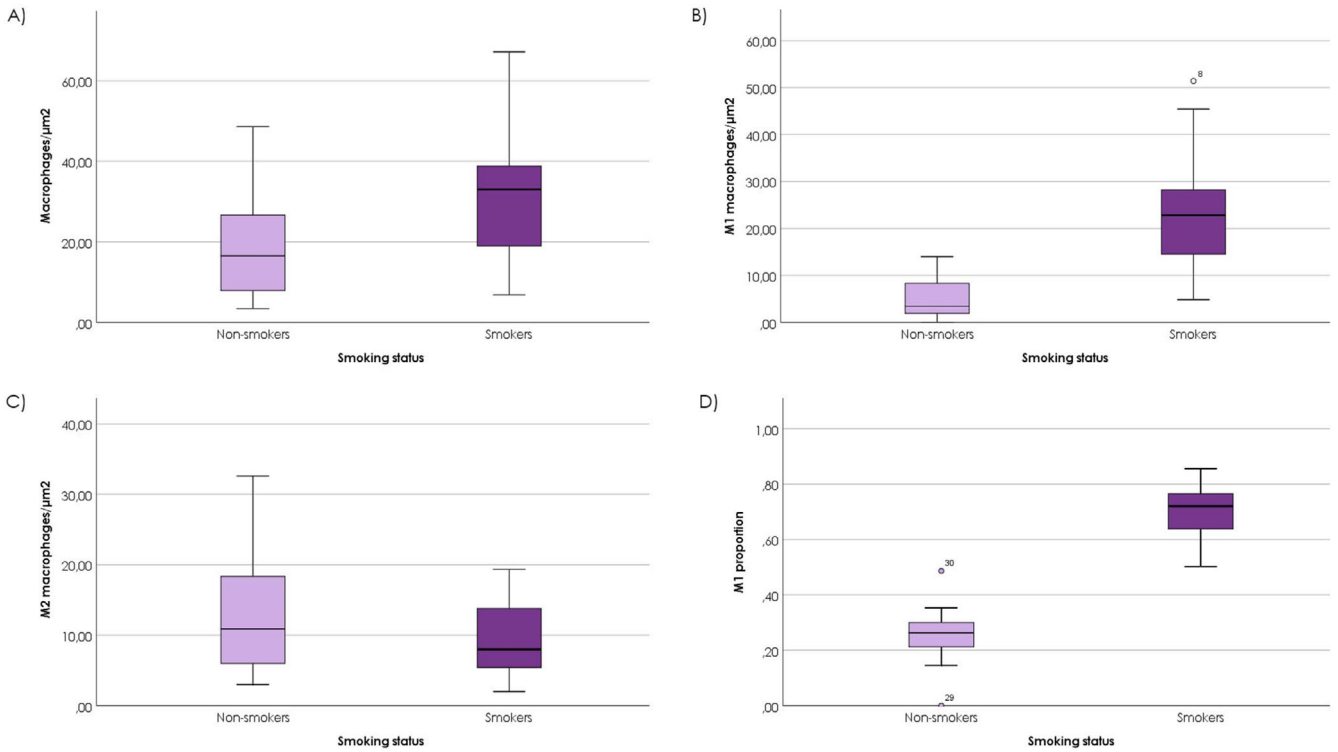

FIGURE 2 | Box plots depicting macrophage quantification across samples: (A) Total number of macrophages per μm^2, (B) Number of M1 macrophages per μm^2, (C) Number of M2 macrophages per μm^2, and (D) Proportion of M1 macrophages relative to the total macrophage population.

TABLE 2 | Macrophage population distribution in smokers and non-smokers.

	Non-smokers ($N=18$)		Smokers ($N=17$)		
	Mean	**SD**	**Mean**	**SD**	***p***
M1 proportion	70.23%	9.65%	25.09%	9.96%	**<0.005**
Macrophages/μm^2	18.79	13.23	32.81	16.32	**0.008**
M1/μm^2	5.04	4.13	23.09	12.37	**<0.005**
M2/μm^2	13.64	9.25	9.68	5.40	0.134

Note: Figures in bold indicate statistical significance ($p<0.05$).

TABLE 3 | Mean M1 proportion according to smoking status, gender, stage of periodontitis, implant position, severity of peri-implantitis.

	Mean	**SD**	***p***
M1 proportion			
Smokers	70.23%	9.65%	**<0.005**
Non smokers	25.09%	9.96%	
Female	40.43%	23.65%	0.21
Male	51.41%	25.21%	
Stage of periodontitis			
II	29.47%	17.41%	**0.01**
III	61.45%	27.61%	
IV	54.31%	22.36%	
Implant position			
Maxilla	57.80%	22.48%	0.208
Mandible	41.40%	24.60%	
Anterior	47.89%	21.59%	0.893
Posterior	46.76%	26.11%	
Severity of peri-implantitis			
Slight	46.04%	23.99%	0.239
Moderate	34.52%	22.03%	
Advanced	54.98%	24.73%	

Note: Figures in bold indicate statistical significance ($p<0.05$).

TABLE 4 | Correlations between M1 proportion and age, mean BOP, mean PPD, mean mPI, MBL max, cotinine, pack × years, function time, and IMI.

	Correlations
	M1 proportion
Age	
Coefficient	−0.377
p	**0.026**
Mean BOP	
Coefficient	0.096
p	0.583
Mean PPD	
Coefficient	0.074
p	0.674
Mean mPI	
Coefficient	0.088
p	0.614
MBL max	
Coefficient	0.127
p	0.466
Cotinine	
Coefficient	0.694
p	**<0.005**
PackxYears	
Coefficient	0.81
p	**<0.005**
Function time	
Coefficient	-0.046
p	0.794
IMI	
Coefficient	0.202
p	0.245

Note: Figures in bold indicate statistical significance ($p<0.05$).

monocytes that migrated from the bloodstream and subsequently differentiated into the M1 phenotype. Indeed, there is evidence that cigarette smoking induces the production of various chemokines and pro-inflammatory cytokines, which promote monocyte adhesion to endothelial cells, facilitating their migration into tissues (Wang et al. 2021). In this regard, MCP-1, one of the most important chemokines involved in monocyte migration, has been shown to correlate positively with smoking duration (Komiyama et al. 2018). However, in the present sample, no association between MCP-1 and smoking status could be demonstrated.

Smokers in this sample were significantly younger than non-smokers. The impact of age-related changes in macrophages and

TABLE 5 | Correlations between cytokine concentration and demographic. clinical. smoking-related. and macrophage-related variables.

	Correlations				
	MCP-1	**TNF-α**	**INF-γ**	**IL-8**	**IL-2**
Age					
Coefficient	0.350	0.323	-0.062	-0.095	−137
p	**0.039**	0.059	0.723	0.587	0.431
Mean BOP					
Coefficient	−0.008	0.082	−0.112	0.344	−0.323
p	0.965	0.639	0.523	**0.043**	0.059
Mean PPD					
Coefficient	0.322	0.252	−0.115	0.570	−0.460
p	0.059	0.143	0.510	**0.000**	**0.005**
Mean mPI					
Coefficient	0.285	0.440	0.442	0.236	0.086
p	0.097	**0.008**	**0.008**	0.172	0.625
MBL max					
Coefficient	0.108	0.192	0.034	0.388	−0.148
p	0.537	0.269	0.848	**0.021**	0.395
Cotinine					
Coefficient	−0.210	−0.296	−0.154	0.242	0.078
p	0.227	0.084	0.377	0.161	0.656
PackxYears					
Coefficient	−0.232	−0.245	−0.035	0.285	0.083
p	0.180	0.156	0.843	0.098	0.637
Function time					
Coefficient	0.034	0.176	0.225	0.320	−0.153
p	0.845	0.312	0.194	0.061	0.379
Stage of periodontitis					
Coefficient	0.102	0.167	0.083	0.153	0.040
p	0.562	0.338	0.634	0.380	0.821
IMI					
Coefficient	0.088	0.002	−0.133	0.244	−0.074
p	0.615	0.991	0.447	0.158	0.672
M1 proportion					
Coefficient	−0.028	−0.106	−0.064	0.148	0.194
p	0.872	0.543	0.716	0.397	0.264
Macrophages/μm²					
Coefficient	−0.011	−0.314	−0.434	−0.026	0.089

(Continues)

TABLE 5 | (Continued)

	Correlations				
	MCP-1	**TNF-α**	**INF-γ**	**IL-8**	**IL-2**
p	0.949	0.066	**0.009**	0.883	0.613
M1/μm²					
Coefficient	−0.057	−0.284	−0.291	0.091	0.153
p	0.747	0.098	0.090	0.601	0.380
M2/μm²					
Coefficient	0.100	−0.263	−0.308	−0.273	0.014
p	0.566	0.127	0.072	0.113	0.936

Note: Figures in bold indicate statistical significance ($p < 0.05$).
Abbreviations: IMI, Implant Mucosal Index; MBL max, Maximum Marginal Bone Loss; Mean BOP, Mean Bleeding on Probing; Mean mPI, Mean Modified Plaque Index; Mean PPD, Mean Probing Pocket Depth.

their potential role in the development of periodontal and peri-implant disease remains under investigation (Clark et al. 2021). A study in non-human primates found increased M1-related gene expression in the gingiva of healthy aged individuals compared to younger controls (Gonzalez et al. 2015). Similarly, RNA sequencing studies have shown heightened pro-inflammatory gene expression (Lafuse et al. 2019), cytokine expression, and M1 macrophage markers (Clark et al. 2020) in older mice compared to younger ones. While the potential influence of age on macrophage polarization cannot be dismissed, the smoker group—despite being younger—exhibited the highest prevalence of M1 macrophages.

Previous studies reported higher levels of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6 in smokers compared to non-smokers (Akram et al. 2018; AlQahtani et al. 2018; ArRejaie et al. 2019; BinShabaib et al. 2019; Taskaldiran et al. 2024). These results contrast with our observation in which the pro-inflammatory cytokine concentrations were mainly associated with clinical and radiological variables rather than smoking. Nonetheless, it is important to note that the present study was not sufficiently powered to detect differences between smokers and non-smokers for this specific outcome. One possible explanation for this finding is that the cytokine concentrations in peri-implant crevicular fluid may not fully capture the inflammatory processes occurring in the peri-implant mucosa. Alternatively, it should be considered that macrophages represent only a small proportion of the overall cellular population within the peri-implant lesion, with plasma cells being the most predominant (Fretwurst et al. 2021). Therefore, the concentration of cytokines in the peri-implant crevicular fluid could be influenced by the presence of other cytokine-secreting cells.

The current study found no significant differences in microbiota richness and diversity between smokers and non-smokers. This contrasts with a previous study, which reported higher α-diversity in smokers compared to non-smokers (Amerio et al. 2022). It is important to note, however, that all participants in the present study had PI, whereas the previous study included smokers and non-smokers with healthy implants, mucositis, and PI. A positive correlation was observed between α-diversity and cotinine concentration, suggesting that smoking may influence microbial diversity once exposure surpasses a certain threshold. However, in contrast to these findings, another study reported that under healthy conditions, smokers had lower α-diversity compared to non-smokers, with microbial diversity further declining as health progressed to PI (Tsigarida et al. 2015).

4.3 | Clinical Implications

The findings from this study highlight the significant impact of smoking on macrophage polarization in PI lesions. The predominance of M1 macrophages in smokers indicates a heightened pro-inflammatory condition, which could exacerbate tissue destruction and complicate treatment outcomes. Dental professionals should emphasize smoking cessation as part of comprehensive periodontal and peri-implant care.

4.4 | Limitations and Strengths of the Study

Several limitations need to be disclosed. First, the cross-sectional design limits the ability to establish a causal link between smoking and macrophage polarization. In addition, the small sample size increases the risk of introducing unforeseen bias, requiring careful interpretation of the results and may constrain the generalizability of the findings. Additionally, only a limited number of cytokines were analyzed in this study. Future research should consider using broader cytokine panels or proteomic analyses (Halstenbach et al. 2023). Another important limitation of this study is the challenge of distinguishing between M1 and M2 macrophages using currently available markers, as these markers can also label other cell types, including fibroblasts, dendritic cells, certain endothelial cell subpopulations, and B cells (Bertani et al. 2017; Galarraga-Vinueza et al. 2020; Garaicoa-Pazmino et al. 2019). Nonetheless, the study offers notable strengths: cotinine levels were measured to assess smoking exposure accurately, and pyrosequencing methods were used to detect potentially uncultivable species.

TABLE 6 | Relative abundances of bacteria in smokers and non-smokers.

	Smokers		Non smokers		
	Mean %	SD	Mean %	SD	*p*
Atopobium parvulum	0.99	2.26	2.04	4.15	0.809
Bifidobacterium longum	1.22	3.89	0.30	0.80	0.564
Campylobacter concisus	0.26	0.92	0.03	0.12	1
Campylobacter curvus	0.00	0.00	0.02	0.08	0.985
Campylobacter gracilis	0.26	0.56	0.25	0.37	0.752
Campylobacter rectus	4.69	8.65	0.92	1.94	0.08
Fusobacterium nucleatum	0.39	1.18	1.04	1.96	0.539
Parvimonas micra	15.94	23.91	12.82	20.76	0.094
Peptostreptococcus stomatis	11.36	26.01	12.18	16.24	0.402
Porphyromonas gingivalis	56.02	40.90	54.68	36.44	0.956
Prevotella histicola	0.61	1.28	0.13	0.34	0.468
Slackia exigua	2.11	2.95	6.65	12.67	0.116
Veillonella parvula	5.43	16.37	9.02	13.37	0.128

5 | Conclusion

In conclusion, despite the limitations of sample size and immunofluorescence analysis, this study suggests that smoking may drive a shift toward M1 macrophages, pointing to a potential mechanism through which smoking could contribute to the onset and/or progression of peri-implantitis. These findings underscore the complex interplay between smoking habits and local immune responses in the context of dental implant health.

Author Contributions

Ettore Amerio: conceptualization, methodology, data curation, formal analysis, investigation, supervision, funding acquisition, writing – original draft, project administration, visualization, software. **Francesco Sparano:** data curation, writing – original draft, investigation, methodology. **Agustín Muñoz-Sanz:** writing – review and editing, resources, methodology, validation, software. **Cristina Valles:** writing – review and editing, methodology, validation, supervision. **Jose Nart:** methodology, validation, writing – review and editing, supervision, funding acquisition. **Alberto Monje:** conceptualization, methodology, writing – review and editing, supervision, validation, funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Aguirre-Zorzano, L. A., R. Estefanía-Fresco, O. Telletxea, and M. Bravo. 2015. "Prevalence of Peri-Implant Inflammatory Disease in Patients With a History of Periodontal Disease Who Receive Supportive Periodontal Therapy." *Clinical Oral Implants Research* 26, no. 11: 1338–1344. https://doi.org/10.1111/clr.12462.

Akram, Z., F. Vohra, I. A. Bukhari, S. A. Sheikh, and F. Javed. 2018. "Clinical and Radiographic Peri-Implant Parameters and Proinflammatory Cytokine Levels Among Cigarette Smokers, Smokeless Tobacco Users, and Nontobacco Users." *Clinical Implant Dentistry and Related Research* 20, no. 1: 76–81. https://doi.org/10.1111/cid.12575.

Almubarak, A., K. K. K. Tanagala, P. N. Papapanou, E. Lalla, and F. Momen-Heravi. 2020. "Disruption of Monocyte and Macrophage Homeostasis in Periodontitis." *Frontiers in Immunology* 11: 330.

AlQahtani, M. A., A. S. Alayad, A. Alshihri, F. O. B. Correa, and Z. Akram. 2018. "Clinical Peri-Implant Parameters and Inflammatory Cytokine Profile Among Smokers of Cigarette, e-Cigarette, and Waterpipe." *Clinical Implant Dentistry and Related Research* 20, no. 6: 1016–1021. https://doi.org/10.1111/cid.12664.

Amerio, E., G. Blasi, C. Valles, et al. 2022. "Impact of Smoking on Peri-Implant Bleeding on Probing." *Clinical Implant Dentistry and Related Research* 24, no. 2: 151–165. https://doi.org/10.1111/cid.13062.

ArRejaie, A. S., K. A. Al-Aali, M. Alrabiah, et al. 2019. "Proinflammatory Cytokine Levels and Peri-Implant Parameters Among Cigarette Smokers, Individuals Vaping Electronic Cigarettes, and Non-Smokers." *Journal of Periodontology* 90, no. 4: 367–374. https://doi.org/10.1002/JPER.18-0045.

Bazzan, E., G. Turato, M. Tinè, et al. 2017. "Dual Polarization of Human Alveolar Macrophages Progressively Increases With Smoking and COPD Severity." *Respiratory Research* 18, no. 1: 40. https://doi.org/10.1186/s12931-017-0522-0.

Berglundh, T., G. Armitage, M. G. Araujo, et al. 2018. "Peri-Implant Diseases and Conditions: Consensus Report of Workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions." *Journal of Clinical Periodontology* 89, no. S1: S313–S318. https://doi.org/10.1002/jper.17-0739.

Berglundh, T., N. U. Zitzmann, and M. Donati. 2011. "Are Peri-Implantitis Lesions Different From Periodontitis Lesions?" *Journal of Clinical Periodontology* 38, no. Suppl 11: 188–202. https://doi.org/10.1111/j.1600-051X.2010.01672.x.

Bertani, F. R., P. Mozetic, M. Fioramonti, et al. 2017. "Classification of M1/M2-Polarized Human Macrophages by Label-Free Hyperspectral Reflectance Confocal Microscopy and Multivariate Analysis." *Scientific Reports* 7, no. 1: 8965. https://doi.org/10.1038/s41598-017-08121-8.

Bianchi, F., V. Le Noci, G. Bernardo, et al. 2024. "Cigarette Smoke Sustains Immunosuppressive Microenvironment Inducing M2 Macrophage Polarization and Viability in Lung Cancer Settings." *PLoS One* 19, no. 5: e0303875. https://doi.org/10.1371/journal.pone.0303875.

BinShabaib, M., A. L. SS, Z. Akram, et al. 2019. "Clinical Periodontal Status and Gingival Crevicular Fluid Cytokine Profile Among Cigarette-Smokers, Electronic-Cigarette Users and Never-Smokers." *Archives of Oral Biology* 102: 212–217. https://doi.org/10.1016/j.archoralbio.2019.05.001.

Canullo, L., D. Peñarrocha-Oltra, U. Covani, D. Botticelli, G. Serino, and M. Penarrocha. 2016. "Clinical and Microbiological Findings in Patients With Peri-Implantitis: A Cross-Sectional Study." *Clinical Oral Implants Research* 27, no. 3: 376–382. https://doi.org/10.1111/clr.12557.

Carcuac, O., and T. Berglundh. 2014. "Composition of Human Peri-Implantitis and Periodontitis Lesions." *Journal of Dental Research* 93, no. 11: 1083–1088. https://doi.org/10.1177/0022034514551754.

Casado, P. L., M. C. Pereira, M. E. L. Duarte, and J. M. Granjeiro. 2013. "History of Chronic Periodontitis Is a High Risk Indicator for Peri-Implant Disease." *Brazilian Dental Journal* 24, no. 2: 136–141. https://doi.org/10.1590/0103-6440201302006.

Clark, D., S. Brazina, F. Yang, et al. 2020. "Age-Related Changes to Macrophages Are Detrimental to Fracture Healing in Mice." *Aging Cell* 19, no. 3: e13112. https://doi.org/10.1111/acel.13112.

Clark, D., E. Kotronia, and S. E. Ramsay. 2021. "Frailty, Aging, and Periodontal Disease: Basic Biologic Considerations." *Periodontology 2000* 87, no. 1: 143–156. https://doi.org/10.1111/prd.12380.

Dalago, H. R., G. Schuldt Filho, M. A. P. Rodrigues, S. Renvert, and M. A. Bianchini. 2017. "Risk Indicators for Peri-Implantitis. A Cross-Sectional Study With 916 Implants." *Clinical Oral Implants Research* 28, no. 2: 144–150. https://doi.org/10.1111/clr.12772.

Daubert, D. M., B. F. Weinstein, S. Bordin, B. G. Leroux, and T. F. Flemming. 2015. "Prevalence and Predictive Factors for Peri-Implant Disease and Implant Failure: A Cross-Sectional Analysis." *Journal of Periodontology* 86, no. 3: 337–347. https://doi.org/10.1902/jop.2014.140438.

de Araújo Nobre, M., A. Mano Azul, E. Rocha, and P. Maló. 2015. "Risk Factors of Peri-Implant Pathology." *European Journal of Oral Sciences* 123, no. 3: 131–139. https://doi.org/10.1111/eos.12185.

Dionigi, C., L. Larsson, O. Carcuac, and T. Berglundh. 2020. "Cellular Expression of DNA Damage/Repair and Reactive Oxygen/Nitrogen Species in Human Periodontitis and Peri-Implantitis Lesions." *Journal of Clinical Periodontology* 47, no. 12: 1466–1475. https://doi.org/10.1111/jcpe.13370.

Dvorak, G., C. Arnhart, S. Heuberer, C. D. Huber, G. Watzek, and R. Gruber. 2011. "Peri-Implantitis and Late Implant Failures in Postmenopausal Women: A Cross-Sectional Study." *Journal of Clinical Periodontology* 38, no. 10: 950–955. https://doi.org/10.1111/j.1600-051X.2011.01772.x.

Eapen, M. S., P. M. Hansbro, K. McAlinden, et al. 2017. "Abnormal M1/M2 Macrophage Phenotype Profiles in the Small Airway Wall and Lumen in Smokers and Chronic Obstructive Pulmonary Disease (COPD)." *Scientific Reports* 7, no. 1: 13392. https://doi.org/10.1038/s41598-017-13888-x.

French, D., D. L. Cochran, and R. Ofec. 2016. "Retrospective Cohort Study of 4,591 Straumann Implants Placed in 2,060 Patients in Private Practice With up to 10-Year Follow-Up: The Relationship Between Crestal Bone Level and Soft Tissue Condition." *International Journal of Oral & Maxillofacial Implants* 31, no. 6: e168–e178. https://doi.org/10.11607/jomi.4932.

Fretwurst, T., C. Garaicoa-Pazmino, K. Nelson, et al. 2020. "Characterization of Macrophages Infiltrating Peri-Implantitis Lesions." *Clinical Oral Implants Research* 31, no. 3: 274–281. https://doi.org/10.1111/clr.13568.

Fretwurst, T., J. Müller, L. Larsson, et al. 2021. "Immunohistological Composition of Peri-Implantitis Affected Tissue Around Ceramic Implants-A Pilot Study." *Journal of Periodontology* 92, no. 4: 571–579. https://doi.org/10.1002/jper.20-0169.

Galarraga-Vinueza, M. E., K. Obreja, A. Ramanauskaite, et al. 2020. "Macrophage Polarization in Peri-Implantitis Lesions." *Clinical Oral Investigations* 25: 2335–2344. https://doi.org/10.1007/s00784-020-03556-2.

Gao, X. R., J. Ge, W. Y. Li, W. C. Zhou, L. Xu, and D. Q. Geng. 2018. "NF-κB/Let-7f-5p/IL-10 Pathway Involves in Wear Particle-Induced Osteolysis by Inducing M1 Macrophage Polarization." *Cell Cycle* 17, no. 17: 2134–2145. https://doi.org/10.1080/15384101.2018.1515549.

Garaicoa-Pazmino, C., T. Fretwurst, C. H. Squarize, et al. 2019. "Characterization of Macrophage Polarization in Periodontal Disease." *Journal of Clinical Periodontology* 46, no. 8: 830–839.

Gonzalez, O. A., M. J. Novak, S. Kirakodu, et al. 2015. "Differential Gene Expression Profiles Reflecting Macrophage Polarization in Aging and Periodontitis Gingival Tissues." *Immunological Investigations* 44, no. 7: 643–664. https://doi.org/10.3109/08820139.2015.1070269.

Halstenbach, T., K. Nelson, G. Iglhaut, O. Schilling, and T. Fretwurst. 2023. "Impact of Peri-Implantitis on the Proteome Biology of Crevicular Fluid: A Pilot Study." *Journal of Periodontology* 94, no. 7: 835–847. https://doi.org/10.1002/jper.22-0461.

Karimi, K., H. Sarir, E. Mortaz, et al. 2006. "Toll-Like Receptor-4 Mediates Cigarette Smoke-Induced Cytokine Production by Human Macrophages." *Respiratory Research* 7, no. 1: 66. https://doi.org/10.1186/1465-9921-7-66.

Koldsland, O. C., A. A. Scheie, and A. M. Aass. 2010. "Prevalence of Peri-Implantitis Related to Severity of the Disease With Different Degrees of Bone Loss." *Journal of Periodontology* 81, no. 2: 231–238. https://doi.org/10.1902/jop.2009.090269.

Koldsland, O. C., A. A. Scheie, and A. M. Aass. 2011. "The Association Between Selected Risk Indicators and Severity of Peri-Implantitis Using Mixed Model Analyses." *Journal of Clinical Periodontology* 38, no. 3: 285–292. https://doi.org/10.1111/j.1600-051X.2010.01659.x.

Komiyama, M., R. Takanabe, K. Ono, et al. 2018. "Association Between Monocyte Chemoattractant Protein-1 and Blood Pressure in Smokers." *Journal of International Medical Research* 46, no. 3: 965–974. https://doi.org/10.1177/0300060517723415.

Lafuse, W. P., M. V. S. Rajaram, Q. Wu, et al. 2019. "Identification of an Increased Alveolar Macrophage Subpopulation in Old Mice That Displays Unique Inflammatory Characteristics and Is Permissive to *Mycobacterium tuberculosis* Infection." *Journal of Immunology* 203, no. 8: 2252–2264. https://doi.org/10.4049/jimmunol.1900495.

Lee, J., V. Taneja, and R. Vassallo. 2012. "Cigarette Smoking and Inflammation: Cellular and Molecular Mechanisms." *Journal of Dental Research* 91, no. 2: 142–149. https://doi.org/10.1177/0022034511421200.

Marrone, A., J. Lasserre, P. Bercy, and M. C. Brecx. 2013. "Prevalence and Risk Factors for Peri-Implant Disease in Belgian Adults." *Clinical Oral Implants Research* 24, no. 8: 934–940. https://doi.org/10.1111/j.1600-0501.2012.02476.x.

Martinez, F. O., A. Sica, A. Mantovani, and M. Locati. 2008. "Macrophage Activation and Polarization." *Frontiers in Bioscience* 13, no. 13: 453–461. https://doi.org/10.2741/2692.

Martinez-Amargant, J., B. de Tapia, A. Pascual, et al. 2023. "Association Between Smoking and Peri-Implant Diseases: A Retrospective Study." *Clinical Oral Implants Research* 34, no. 10: 1127–1140. https://doi.org/10.1111/clr.14147.

Máximo, M. B., A. C. de Mendonça, J. F. Alves, S. C. Cortelli, D. C. Peruzzo, and P. M. Duarte. 2008. "Peri-Implant Diseases May Be Associated With Increased Time Loading and Generalized Periodontal Bone Loss: Preliminary Results." *Journal of Oral Implantology* 34, no. 5: 268–273. https://doi.org/10.1563/1548-1336(2008)34[269:PDMBAW]2.0.CO;2.

Miyajima, S.-i., K. Naruse, Y. Kobayashi, et al. 2014. "Periodontitis-Activated Monocytes/Macrophages Cause Aortic Inflammation." *Scientific Reports* 4, no. 1: 1–9.

Mombelli, A., M. A. van Oosten, E. Schurch, and N. P. Land. 1987. "The Microbiota Associated With Successful or Failing Osseointegrated Titanium Implants." *Oral Microbiology and Immunology* 2, no. 4: 145–151. https://doi.org/10.1111/j.1399-302x.1987.tb00298.x.

Monje, A., R. Pons, A. Insua, J. Nart, H. L. Wang, and F. Schwarz. 2019. "Morphology and Severity of Peri-Implantitis Bone Defects." *Clinical Implant Dentistry and Related Research* 21, no. 4: 635–643. https://doi.org/10.1111/cid.12791.

Negri, B. M., S. P. Pimentel, M. Z. Casati, F. R. Cirano, R. C. Casarin, and F. V. Ribeiro. 2016. "Impact of a Chronic Smoking Habit on the Osteo-Immunoinflammatory Mediators in the Peri-Implant Fluid of Clinically Healthy Dental Implants." *Archives of Oral Biology* 70: 55–61. https://doi.org/10.1016/j.archoralbio.2016.05.014.

Parisi, L., E. Gini, D. Baci, et al. 2018. "Macrophage Polarization in Chronic Inflammatory Diseases: Killers or Builders?" *Journal of Immunology Research* 2018: 8917804.

Park, E. J., H. S. Lee, S. J. Lee, et al. 2018. "Cigarette Smoke Condensate May Disturb Immune Function With Apoptotic Cell Death by Impairing Function of Organelles in Alveolar Macrophages." *Toxicology In Vitro* 52: 351–364. https://doi.org/10.1016/j.tiv.2018.07.014.

Phaybouth, V., S. Z. Wang, J. A. Hutt, J. D. McDonald, K. S. Harrod, and E. G. Barrett. 2006. "Cigarette Smoke Suppresses Th1 Cytokine Production and Increases RSV Expression in a Neonatal Model." *American Journal of Physiology. Lung Cellular and Molecular Physiology* 290, no. 2: L222–L231. https://doi.org/10.1152/ajplung.00148.2005.

Rakic, M., P. Galindo-Moreno, A. Monje, et al. 2018. "How Frequent Does Peri-Implantitis Occur? A Systematic Review and Meta-Analysis." *Clinical Oral Investigations* 22, no. 4: 1805–1816. https://doi.org/10.1007/s00784-017-2276-y.

Reis, I., G. do Amaral, M. A. Hassan, et al. 2023. "The Influence of Smoking on the Incidence of Peri-Implantitis: A Systematic Review and Meta-Analysis." *Clinical Oral Implants Research* 34, no. 6: 543–554. https://doi.org/10.1111/clr.14066.

Renvert, S., A. Aghazadeh, H. Hallström, and G. R. Persson. 2014. "Factors Related to Peri-Implantitis – a Retrospective Study." *Clinical Oral Implants Research* 25, no. 4: 522–529. https://doi.org/10.1111/clr.12208.

Rinke, S., S. Ohl, D. Ziebolz, K. Lange, and P. Eickholz. 2011. "Prevalence of Periimplant Disease in Partially Edentulous Patients: A Practice-Based Cross-Sectional Study." *Clinical Oral Implants Research* 22, no. 8: 826–833. https://doi.org/10.1111/j.1600-0501.2010.02061.x.

Rokn, A., H. Aslroosta, S. Akbari, H. Najafi, F. Zayeri, and K. Hashemi. 2017. "Prevalence of Peri-Implantitis in Patients Not Participating in Well-Designed Supportive Periodontal Treatments: A Cross-Sectional Study." *Clinical Oral Implants Research* 28, no. 3: 314–319. https://doi.org/10.1111/clr.12800.

Roos-Jansåker, A.-M., C. Lindahl, H. Renvert, and S. Renvert. 2006a. "Nine-to Fourteen-Year Follow-Up of Implant Treatment. Part II: Presence of Peri-Implant Lesions." *Journal of Clinical Periodontology* 33, no. 4: 290–295. https://doi.org/10.1111/j.1600-051X.2006.00906.x.

Roos-Jansåker, A.-M., H. Renvert, C. Lindahl, and S. Renvert. 2006b. "Nine- To Fourteen-Year Follow-Up of Implant Treatment. Part III: Factors Associated With Peri-Implant Lesions." *Journal of Clinical Periodontology* 33, no. 4: 296–301. https://doi.org/10.1111/j.1600-051X.2006.00908.x.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. "NIH Image to ImageJ: 25 Years of Image Analysis." *Nature Methods* 9, no. 7: 671–675. https://doi.org/10.1038/nmeth.2089.

Schwarz, F., J. Derks, A. Monje, and H.-L. Wang. 2018. "Peri-implantitis." *Journal of Clinical Periodontology* 45, no. Suppl 20: S246–S266. https://doi.org/10.1111/jcpe.12954.

Schwarz, F., G. John, A. Schmucker, N. Sahm, and J. Becker. 2017. "Combined Surgical Therapy of Advanced Peri-Implantitis Evaluating Two Methods of Surface Decontamination: A 7-Year Follow-Up Observation." *Journal of Clinical Periodontology* 44, no. 3: 337–342. https://doi.org/10.1111/jcpe.12648.

Shaykhiev, R., A. Krause, J. Salit, et al. 2009. "Smoking-Dependent Reprogramming of Alveolar Macrophage Polarization: Implication for Pathogenesis of Chronic Obstructive Pulmonary Disease." *Journal of Immunology* 183, no. 4: 2867–2883. https://doi.org/10.4049/jimmunol.0900473.

Stoger, J. L., M. J. Gijbels, S. van der Velden, et al. 2012. "Distribution of Macrophage Polarization Markers in Human Atherosclerosis." *Atherosclerosis* 225, no. 2: 461–468. https://doi.org/10.1016/j.atherosclerosis.2012.09.013.

Taskaldiran, E. S., G. Tuter, A. A. Yucel, and M. Yaman. 2024. "Effects of Smoking on the Salivary and GCF Levels of IL-17 and IL-35 in Periodontitis." *Odontology* 112, no. 2: 616–623. https://doi.org/10.1007/s10266-023-00843-8.

Thomas, W. R., P. G. Holt, and D. Keast. 1978. "Cigarette Smoke and Phagocyte Function: Effect of Chronic Exposure In Vivo and Acute Exposure In Vitro." *Infection and Immunity* 20, no. 2: 468–475. https://doi.org/10.1128/iai.20.2.468-475.1978.

Tsigarida, A. A., S. M. Dabdoub, H. N. Nagaraja, and P. S. Kumar. 2015. "The Influence of Smoking on the Peri-Implant Microbiome." *Journal of Dental Research* 94, no. 9: 1202–1217. https://doi.org/10.1177/0022034515590581.

Wang, W., T. Zhao, K. Geng, G. Yuan, Y. Chen, and Y. Xu. 2021. "Smoking and the Pathophysiology of Peripheral Artery Disease." *Frontiers in Cardiovascular Medicine* 8: 704106. https://doi.org/10.3389/fcvm.2021.704106.

Yang, D. C., and C. H. Chen. 2018. "Cigarette Smoking-Mediated Macrophage Reprogramming: Mechanistic Insights and Therapeutic Implications." *Journal of Nature and Science* 4, no. 11: e539.

Yu, T., L. Zhao, X. Huang, et al. 2016. "Enhanced Activity of the Macrophage M1/M2 Phenotypes and Phenotypic Switch to M1 in Periodontal Infection." *Journal of Periodontology* 87, no. 9: 1092–1102. https://doi.org/10.1902/jop.2016.160081.

Yuan, F., X. Fu, H. Shi, G. Chen, P. Dong, and W. Zhang. 2014. "Induction of Murine Macrophage M2 Polarization by Cigarette Smoke Extract via the JAK2/STAT3 Pathway." *PLoS One* 9, no. 9: e107063. https://doi.org/10.1371/journal.pone.0107063.

Zhou, L. N., C. S. Bi, L. N. Gao, Y. An, F. Chen, and F. M. Chen. 2019. "Macrophage Polarization in Human Gingival Tissue in Response to Periodontal Disease." *Oral Diseases* 25, no. 1: 265–273.

Zhuang, Z., S. Yoshizawa-Smith, A. Glowacki, et al. 2019. "Induction of M2 Macrophages Prevents Bone Loss in Murine Periodontitis Models." *Journal of Dental Research* 98, no. 2: 200–208.