

Supplemental Material

Distinct Microbial Signatures Among Periodontal Profile Classes

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Methods

Study population and disease classification

The ARIC study, supported by the National Heart, Lung, and Blood Institute, is a prospective investigation of the etiology and natural history of atherosclerosis and clinical cardiovascular disease in 4 US communities (Jackson, Miss; Washington County, Maryland; suburban Minneapolis, Minn; and Forsyth County, North Carolina). A sample of 15,792 community-dwelling residents aged 45 to 64 years at baseline took part in an evaluation of cardiovascular risk factors and sequelae. The dataset is publicly available upon request to the parent study (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000280.v6.p1).

The Dental ARIC (DARIC) was an ancillary (supplementary) study funded by the NIDCR conducted at ARIC visit 4 (1996-1998). The DARIC cohort study comprised 6,793 dentate individuals living in the four United States communities described above. Demographics and medical history were recorded. The Dental ARIC consisted of an oral examination, with collection of the following clinical measures: Probing depth (PD) and cemento-enamel junction (CEJ) - determined with a UNC-15 periodontal probe at 6 sites/tooth; bleeding on probing (BOP), assessed after probing each tooth surfaces. Gingival index, Loe & Silness – determined as 0=normal gingiva; 1=mild inflammation: slight change in color, slight edema, no BOP; 2=moderate inflammation: redness, edema, and glazing, BOP. 3=severe inflammation: marked redness and edema, ulceration, tendency to bleed spontaneously. Plaque score - determined

on a 0-3 scale. The dental examiners were calibrated against a standard examiner, as well as each other.

The current study consisted of a subset of 1,450 adult dentate participants of the DARIC study that had biofilm collection and serum collection in addition to the clinical measurements described above. Individuals requiring antibiotic prophylaxis were excluded from the study.

Collection and analysis of Bacterial biofilm composition and Serum IgG Antibody Titers

Plaque and serum samples were collected and evaluated according to methods previously described (Beck et al 2005). Briefly, four plaque samples were obtained from the mesial surface of all first molars using a sterile curette. If the tooth was missing, sampling occurred on the mesial surface of the nearest most posterior adjacent tooth. The site was isolated with a cotton roll and removed of gross supragingival plaque or debris if present and gently dried with sterile gauze or air stream. Plaque samples will be taken by placing a sterile curette to the base of each pocket and, with a single stroke, pressing against the tooth at the depth of the pocket, removing a sample for transfer to the storage buffer. Supragingival biofilm was not collected. The biofilm sample was placed into a tube containing 0.1 mL sterile TE buffer. Following sample collection, 0.1 mL of 0.5 M NaOH (sterile) was added using the plunger-style dispenser, mixed well, and stored at -80°C for further analysis. In the current study, one biofilm sample from each individual was assayed by DNA-DNA checkerboard for the eight periodontal pathogens. Total counts reflect a sum of these targeted pathogens for each individual. Serum samples were collected and frozen at -80°C until further analysis. The samples were assayed for IgG antibody levels using the checkerboard immunoblotting technique described by Beck et al. 2005.

Supplemental Table 1. Periodontal Profile Class definitions according to different references.

Beck et al 2020		Morelli et al 2017	
Stage	Description	Class	Description
I	Health	A	Health
II	Mild	B	Mild Disease
III	Moderate	E	Posterior Disease
IV	Severe Disease	G	Severe Disease
V	Mild Tooth Loss (TL)/High Gingival Index (GI)	C	High gingival index (GI) scores
VI	Moderate (Mod) Tooth Loss (TL)/Reduced (Red) Periodontium (Perio)	D	Tooth Loss
VII	Severe Tooth Loss (TL)	F	Lower Incisors

Supplemental Table 2: Number of subjects by Periodontal Profile Classes (PPC) and World Workshop 2017 (WW17 modified*) periodontal disease classification.

	WW17 Stage I	WW17 Stage II	WW17 Stage III	WW17 Stage IV	Total (PPC)
PPC-Stage I (Health)	34	265	72	13	384
PPC-Stage II (Mild)	10	141	74	10	235
PPC-Stage III (Moderate)	0	34	163	19	216
PPC-Stage IV (Severe)	0	13	90	20	123
PPC-Stage V (Mild TL/High GI)	0	30	23	70	123
PPC-Stage VI (Red Perio)	1	16	17	140	174
PPC-Stage VII (Severe TL)	0	0	0	195	195
Total (WW17)	45	499	439	467	1450

*The clinical data used to model the World Workshop grades does not contain longitudinal measures of clinical attachment loss or radiographic bone loss; the stages were defined by clinical attachment levels and defined as a modified version, described previously (Beck et al. 2019).

TL= tooth loss; GI= gingival index.

Supplemental Table 3A. Mean (se) adjusted* log microbial counts by the Centers for Disease Control/American Academy of Periodontology definition of disease.

Bacterial biofilm	Health n=158	Mild n=431	Moderate n=588	Severe n=273	p-value
<i>P. intermedia</i>	2.87 (0.16)	2.82 (0.10)	2.70 (0.08)	2.78 (0.13)	1.00
<i>P. nigrescens</i>	2.69 (0.15)	2.76 (0.09)	2.89 (0.08)	2.97 (0.12)	1.00
<i>F. nucleatum</i>	2.91 (0.16)	2.98 (0.10)	3.06 (0.08)	3.04 (0.13)	1.00
<i>C. rectus</i>	2.80 (0.14)	2.75 (0.08)	2.91 (0.07)	2.96 (0.11)	1.00
<i>P. gingivalis</i>	2.19 (0.13)	2.32 (0.08)	2.40 (0.07)	2.68 (0.10)	0.11
<i>T. forsythia</i>	2.70 (0.13)	2.41 (0.08)	2.55 (0.07)	2.79 (0.10)	0.17
<i>T. denticola</i>	2.74 (0.14)	2.66 (0.08)	2.73 (0.07)	2.84 (0.11)	1.00
<i>A. actinomycetemcomitar</i>	2.73 (0.13)	2.65 (0.08)	2.78 (0.06)	2.74 (0.10)	1.00

Supplemental Table 3B. Mean (se) adjusted* log microbial counts by World Workshop 2017 definition of disease (modified)**.

Bacterial biofilm	Stage I N=45	Stage II N=499	Stage III N=439	Stage IV N=467	p-value
<i>P. intermedia</i>	2.67 (0.30)	2.86 (0.09)	2.61 (0.10)	2.83 (0.10)	1.00
<i>P. nigrescens</i>	2.34 (0.29)	2.91 (0.09)	2.83 (0.09)	2.85 (0.10)	1.00
<i>F. nucleatum</i>	1.94 (0.30)	3.14 (0.09)	3.02 (0.10)	2.98 (0.10)	0.02
<i>C. rectus</i>	2.67 (0.25)	2.80 (0.08)	2.83 (0.08)	2.96 (0.08)	1.00
<i>P. gingivalis</i>	1.61 (0.24)	2.44 (0.08)	2.43 (0.08)	2.42 (0.08)	0.09
<i>T. forsythia</i>	2.45 (0.24)	2.45 (0.08)	2.57 (0.08)	2.70 (0.08)	1.00
<i>T. denticola</i>	2.59 (0.26)	2.72 (0.08)	2.72 (0.08)	2.77 (0.09)	1.00
<i>A. actinomycetemcomitans</i>	2.21 (0.23)	2.65 (0.07)	2.77 (0.08)	2.82 (0.08)	0.57

p-values based upon general linear models. *Data adjusted for race/center, age, sex, diabetes, body mass index, smoking (3-levels:current smoker, former smoker, non-smoker), education (3-levels: basic, intermediate, advanced) and dental utilization (frequent dental visits, episodic dental visits). p-values based on general linear models.

** The clinical data used to model the World Workshop grades does not contain longitudinal measures of clinical attachment loss or radiographic bone loss; the stages were defined by clinical attachment levels and defined as a modified version, described previously (Beck et al. 2019).

Color-coding system applied across all periodontal classes in accordance to levels of microorganisms (separate coding/row): green=low, yellow=mild, orange=moderate, red=high levels.

Supplemental Table 4A. Mean (se) adjusted* log microbial IgG levels stratified by the Centers for Disease Control/American Academy of Periodontology definition of disease (n=1,450).

	Health	Mild	Moderate	Severe	p-value
<i>P. intermedia</i>	1.38 (0.06)	1.38 (0.03)	1.52 (0.03)	1.59 (0.04)	0.004
<i>P. nigrescens</i>	1.73 (0.05)	1.73 (0.03)	1.78 (0.03)	1.76 (0.04)	1.00
<i>F. nucleatum</i>	0.85 (0.05)	0.85 (0.03)	0.85 (0.03)	0.82 (0.04)	1.00
<i>C. rectus</i>	1.04 (0.05)	1.03 (0.03)	1.16 (0.03)	1.21 (0.04)	0.009
<i>P. gingivalis</i>	0.98 (0.06)	0.97 (0.03)	1.23 (0.03)	1.52 (0.04)	<0.0001
<i>T. forsythia</i>	1.29 (0.05)	1.26 (0.03)	1.33 (0.02)	1.35 (0.04)	1.00
<i>T. denticola</i>	1.30 (0.04)	1.28 (0.02)	1.35 (0.02)	1.35 (0.03)	0.63
<i>A. actinomycetemcomitans</i>	1.76 (0.05)	1.78 (0.03)	1.87 (0.02)	1.91 (0.04)	0.10

Supplemental Table 4B. Mean (se) adjusted* log microbial IgG levels stratified by WW17 periodontal disease classification (n=1,450).

	Stage I	Stage II	Stage III	Stage IV	p-value
<i>P. intermedia</i>	1.39 (0.10)	1.44 (0.03)	1.56 (0.03)	1.45 (0.03)	0.24
<i>P. nigrescens</i>	1.82 (0.10)	1.75 (0.03)	1.80 (0.03)	1.72 (0.03)	1.00
<i>F. nucleatum</i>	0.85 (0.10)	0.83 (0.03)	0.85 (0.03)	0.85 (0.03)	1.00
<i>C. rectus</i>	1.06 (0.10)	1.06 (0.03)	1.20 (0.03)	1.10 (0.03)	0.09
<i>P. gingivalis</i>	0.95 (0.11)	1.00 (0.03)	1.36 (0.03)	1.22 (0.04)	<0.0001
<i>T. forsythia</i>	1.36 (0.09)	1.30 (0.03)	1.33 (0.03)	1.29 (0.03)	1.00
<i>T. denticola</i>	1.29 (0.07)	1.31 (0.02)	1.36 (0.02)	1.31 (0.02)	1.00
<i>A. actinomycetemcomitans</i>	1.82 (0.09)	1.81 (0.03)	1.90 (0.03)	1.82 (0.03)	0.94

p-values based upon general linear models. Bold indicates significant using PPC-Stage I as reference (Bonferroni corrected). *Data adjusted for race/center, age, sex, diabetes, body mass index, smoking (3-levels: current smoker, former smoker, non-smoker), education (3-levels: basic, intermediate, advanced) and dental utilization (frequent dental visits, episodic dental visits). p-values based upon general linear models.

Color-coding system applied across all periodontal classes in accordance to systemic antibody levels (separate coding/row): green=low, yellow=mild, orange=moderate, red=high levels.