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Periodontal inflammatory burden correlates with C-reactive protein serum level

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Abstract

Objective. The aim of study was to present a new method for evaluation of the periodontal inflammatory burden, to apply the method to the adult population and to correlate it with serum levels of C-reactive protein (CRP). ***Materials and methods.*** On 515 extracted teeth was measured the neck circumferences (NC). The average values of the NC were obtained for 16 male and 16 female individual tooth types. In the clinical part of this study 238 dentate subjects were included. The subgingival area, inflamed area and periodontal wound size were calculated from NC, probing depth and BOP. The sum of the inflamed and ulcerated subgingival areas of all teeth represented the total periodontal inflammatory burden of an individual. Serum levels of CRP were measured by immunochemical method. ***Results.*** The average subgingival area in 238 subjects was calculated to be 13.11 ± 6.35 cm^2 and inflammatory burden area 9.25 ± 5.57 cm^2. The periodontal bleeding wound ($p < 0.05$) was significantly larger in men. The increased serum levels of CRP correlated with periodontal inflammatory burden ($p < 0.05$). ***Conclusions.*** This new method quantifies the inflammatory burden caused by periodontal disease. The size of the inflammatory burden is correlated with increased serum levels of CRP.

Key Words: *C-reactive protein, inflammatory burden, periodontal wound, tooth neck circumferences*

Introduction

Periodontal diseases are infections which are characterized by inflammation and destruction of supporting tissues of the affected teeth. Although the data indicate a possible trend of lower prevalence of periodontitis in recent years [1], periodontitis still affects 5–15% of any population and is a major cause of tooth loss [2,3]. In addition, periodontitis may represent a potential risk factor for many systemic diseases and conditions including cardiovascular disease and stroke [4–6], diabetes [7,8], respiratory diseases [9] and pre-term low birth weight [10–13]. The causal association between periodontitis and cardiovascular disease is still not completely identified. It is possible that elevated levels of CRP in periodontitis are partially responsible for the relationship between periodontitis and cardiovascular disease [14].

C-reactive protein is an acute-phase reactant produced by hepatocytes. The concentration of CRP increases 4–6 h after acute tissue injury or inflammation and declines rapidly with resolution of the injurious process [15]. In healthy persons, normal CRP levels are generally considered to be <3 mg/L. Low-grade inflammation can produce minor elevations of CRP in the 3–10 mg/L range. C-reactive protein levels >10 mg/L may suggest the presence of an underlying inflammatory disease [16]. It was demonstrated that C-reactive protein (CRP) is one of the most predictive biomarkers of systemic inflammation and it's elevated levels represent a risk predictor for cardiovascular diseases [17–19]. hs-CRP assay reports levels as low as 0.1 mg/L and is thus used in CVD risk stratification. Patients with hs-CRP levels <1 mg/L are categorized as having lower relative risk for cardiovascular events. Those with levels of 1–3 mg/L are at intermediate risk, and those with levels >3 mg/L are at higher relative risk [20].

Besides its importance as a biomarker, the significance of extra-hepatic CRP production by the endothelium of atherosclerotic plaques, smooth muscle cells and infiltrated macrophages [21] and its potent biological activities to induce adhesion molecules and

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chemokines at local sites has been shown [22,23]. However, a major source of CRP remains hepatocytes, which have been shown to be induced by stimulation of interleukin (IL)-1β and IL-6 [21].

The preliminary studies showed gene expression of CRP in periodontitis tissues, indicating the existing role of extra-hepatic CRP production in gingival tissues in the pathogenesis of periodontitis [24]. It was found that the expression of CRP mRNA was higher in periodontitis tissues compared to those with gingivitis. CRP exerts activation of a complementary system [25] and induces adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin in human endothelial cells *in vitro* [26]. Once leukocytes adhere to the endothelium, CRP promotes the release of monocyte chemoattractant protein 1, a key chemoattractant chemokine, which facilitates leukocyte transmigration through the endothelium [27]. Besides those fundamental inflammatory responses, CRP induces matrix metalloproteinase (MMP) 1 and MMP9 in human endothelial cells [28], suggesting that CRP might have an important role in the destruction of connective tissues in periodontal inflammation. Therefore, it is assumed that the local expression of CRP in gingival tissues may play important roles in the development and progression of periodontitis.

Maekawa et al. [24] showed that human arterial epithelial cells were also responsible for producing CRP due to infection by oral pathogens. Periodontopathic bacteria which invade gingival tissues may interact directly with arterial endothelial cells, resulting in local production of CRP [29,30].

In spite of the substantial production of CRP by endothelial cells, it is difficult to imagine that systemic elevation of CRP in periodontitis patients is solely attributable to the local production of CRP in periodontitis lesions because the level produced by endothelial cells in response to pro-inflammatory cytokines is much lower compared with that seen in blood. Therefore, it is speculated that the CRP produced in the local periodontitis environment may modulate the inflammatory response on-site.

Elevated CRP levels in serum have been reported in periodontitis patients [14]; however, the mechanism for the elevation of CRP in periodontitis patients has not been clearly demonstrated. Several studies have reported that severe periodontal infection results in increased IL-6 synthesis in the liver, to produce CRP [31–33]. Yamaguchi et al. [34] hypothesized that the source of IL-6 in severe periodontitis patients might be either Kupfer cells in the liver and/or adipocytes, in the case of fatty liver.

The hypothesis is that periodontitis could represent a source of bacteria and their components that can be released into the bloodstream during tooth extraction [35], endodontic treatment [36], scaling and root planning [37,38], subgingival irrigation [39] and even tooth brushing [40] and mastication [41]. The risk of transient bacteriemia induced by these procedures has been shown to correlate with the level of gingival inflammation [40]. Secondly, cells within the connective tissue underlying the periodontal pockets may secrete inflammatory mediators such as CRP, plasminogen activator 1 and fibrinogen [42], which in turn may lead to systemic problems [43]. A biological model was proposed [44] which suggests that periodontitis as a risk factor for other diseases represents an inflammatory burden by eliciting bacteriemia and raised levels of inflammatory mediators, one of which is CRP or cross-reactivity leading to autoimmune reactions.

Thus, periodontal pockets represent a subgingival inflamed or ulcerated area that is infected and is almost permanently submitted to mechanical forces. The total surface area of the periodontal niches in a patient with severe periodontitis can amount to several square centimeters, in certain studies the size was estimated to be equivalent to that of the palm of the hand or that of the ventral surface of the forearm [45,46]. However, Hujoel et al. [47] reported that the dentogingival epithelial surface area among individuals with periodontitis is considerably smaller and ranges from 8–20 cm^2. More recently, Nesse et al. [44] quantified the periodontal inflamed surface area and calculated it to range from 0.3 cm^2 in healthy individuals to 39 cm^2 in patients with severe generalized periodontitis. Several studies have examined the association between periodontitis determined by probing depth, attachment loss and bone loss and CRP serum level [14]. A meta-analysis of studies demonstrated that CRP level in patients with periodontitis was 1.56 mg/l higher than in controls [14].

The aim of this study was to develop a new method for measuring the size of the total subgingival area, inflamed subgingival area and bleeding periodontal wound, to apply this method to the adult population and to correlate the periodontal inflammatory burden size to the levels of serum CRP.

Materials and methods

Assessment of the average teeth neck circumferences

In the first *in vitro* part of the study we included 515 teeth extracted for various reasons at the Department of Oral Surgery. Two hundred and forty-four teeth obtained from men and 271 from women were divided by tooth type, putting together the left and right teeth of the same tooth type, into 16 groups for men and 16 groups for women. Every tooth type group contained at least 10 teeth. We cleaned the extracted teeth with curettes in order to remove the remaining attached soft tissues and calculus. For measuring the circumferences of the teeth necks we lined the dental floss on the cemento-enamel

junction, cut the dental floss with scissors and taped the piece of cut floss on a sheet of paper. We finally had 16 sheets of paper with taped flosses for men and 16 sheets for women. The length of the flosses was then measured by an optical scanner (ScanJet 6300C, Hewlett Packard, USA) with a resolution of 6000 pixels/inch and program for image analysis (UTHSCSA Image Tool for Windows Version 2.00, University of Texas Health Science Center, San Antonio, TX). After receiving the values for each individual tooth we calculated the average cervical circumference for each tooth type. Finally we obtained 16 values for individual tooth type for men and 16 values for individual tooth type for women.

Evaluation of the periodontal inflammatory burden

In the second clinical part of the study we included 238 dentate Ljubljana citizens who participated in our two previous epidemiological surveys of periodontal treatment needs 20 and 10 years ago [48,49]. The results of the periodontal treatment needs of the third survey are reported elsewhere [50]. Among 238 subjects there were 100 men and 138 women. They were divided according to their age into six groups: 35-, 45-, 55-, 65-, 75- and 85-year-old subjects. On all the subjects we performed a clinical oral examination including the measuring of probing depths and bleeding on probing at six sites around each tooth.

To estimate the subgingival area, periodontal wound size and inflammatory burden we utilized measures of average teeth neck circumferences by tooth type, probing depth (PD) and bleeding on probing (BOP). The subgingival area for each tooth was calculated by multiplying 1/6th of the average cervical circumference of the tooth with each of the six measurements of probing depth. The sum of subgingival areas of all present teeth in a subject represented the total subgingival area of an individual.

We further divided the subgingival area into four categories: (a) healthy subgingival area (PD ≤ 2 mm, BOP-), (b) shallow bleeding wound (PD ≤ 2 mm, BOP+), (c) deep non-bleeding area (PD > 2 mm, BOP-) and (d) deep bleeding wound (PD > 2 mm, BOP+) (Table I). Categories (b), (c) and (d) together (b + c + d) represented the inflammatory burden, while categories (b) and (d) only (b + d) represented the total bleeding periodontal wound of an individual.

Table I. Graphic presentation of subgingival area.

		Probing depth (PD) in mm	
		≤2	>2
Bleeding on probing (BOP)	–	a	c
	+	b	d

a = healthy subgingival area; b = shallow bleeding wound; c = deep non-bleeding area; d = deep bleeding wound; a + b + c + d = total subgingival area; b + c + d = total inflammatory burden; b + d = total bleeding peridontal wound.

Measurements of hsCRP serum level

From each of our subjects we collected blood samples from their fingertips. Different studies have demonstrated that CRP is increased in diabetic patients [51], patients with hypertension [52], patients with rheumatoid arthritis [53] and obese subjects [54], while it is decreased in smokers [55] and patients on statin therapy [56]. As we found 102 patients with either diabetes, hypertension, rheumatoid arthritis, patients on statin therapy or a combination of any of the mentioned diseases/therapies, 14 obese subjects (BMI ≥ 30) and 29 smokers, all of these patients were excluded from the study. Our test group finally consisted of 92 subjects, 34 men and 58 women.

The serum level of CRP was measured in all patients by immunochemical method with chemiluminescence detection on an IMMULITE analyzer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany), which enables measurements of very low concentrations of CRP (high-sensitivity CRP). The hs-CRP detection method on the IMMULITE automated analyzer is a two-site chemiluminescent enzyme immunometric assay with a detection limit of 0.10 mg/l and a measuring range of 0.10–500 mg/l.

The study protocol was reviewed and approved by the National Medical Ethics Committee of the Republic of Slovenia. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000, and all participants signed informed consent forms.

Statistical analysis

For purposes of statistical analysis, the clinical variables were tooth neck circumferences, healthy subgingival area, shallow bleeding wound, deep bleeding and deep non-bleeding wound as well as total subgingival area, total inflammatory burden and total bleeding wound. All statistical procedures were based on a level of significance of 5% ($p < 0.05$) and a power of 80%. Summary statistics were calculated for all clinical variables. Statistical analysis was performed using Student's *t*-test for comparison of tooth neck circumferences between men and women and by analysis of variance followed by Tukey HSD test for comparison of periodontal wound areas between age and gender groups. Non-parametric linear regression analysis was used to evaluate the relationship between periodontal inflammatory burden and

hsCRP using Spearman's rank correlation and Fisher's r-to-z transformation method to compare Spearman correlation coefficients for men and women.

Results

We found that in all tooth type groups average values of circumferences of teeth necks were larger in men than in women in the range of 0.18 mm for lower first premolars to 2.47 mm for upper first molars. The total length of all circumferences of all teeth was also larger in men (808.72 ± 18.91 mm) than in women (770.00 ± 18.61 mm) ($p < 0.05$). The greatest circumferences of teeth necks in men in the upper and lower jaw belonged to the first molars, the smallest circumferences in the upper jaw belonged to the lateral incisors and in the lower jaw to the central incisors. In women, the largest circumferences of teeth necks were found in both jaws in second molars, the smallest circumferences in the upper jaw belonged to the lateral incisors and in the lower jaw to the central incisors (Table II).

The results demonstrate that the size of average total subgingival area (a + b + c + d) among Ljubljana 35–85 year old citizens was 13.11 ± 6.35 cm^2 (range: 0.30 – 29.82 cm^2) (Table III). Calculating the separate categories of the subgingival area in our population we found that the healthy subgingival area (a) represented 29.5% out of the total subgingival area (average: 3.86 ± 2.86 cm^2, range: 0.30–12.07 cm^2), while the other 70.5% (average: 9.25 ± 5.57 cm^2, range: 0.30–28.71 cm^2) was represented by the total inflammatory burden (b + c + d). Out of the total inflammatory burden, 21.6% (average: 2.84 ± 2.91 cm^2, range: 0.30–17.42 cm^2) was represented by total periodontal bleeding wound (b + d) and 48.9% (average: 6.40 ± 4.31 cm^2, range: 0.30–26.90 cm^2) was represented by the deep non-bleeding area (c) (Figure 1).

The average total subgingival area was the largest in the 35-year-old population (19.53 cm^2) and decreased with age, dropping to 7.30 cm^2 in the 85-year-old population. A similar trend was found in average healthy subgingival area ranging from 6.10 cm^2 in 35-year-olds to 2.20 cm^2 in 85-year-olds and in shallow bleeding wound ranging from 0.54 cm^2 in the 35-year-old population to 0.10 cm^2 in the 85-year-old population. Deep non-bleeding subgingival area ranged from 9.58 cm^2 in 35-year-olds to 3.54 cm^2 in 85-year-olds. Deep periodontal wound size was the largest in 55-year-olds (3.73 cm^2) and the smallest in 75-year-olds (1.23 cm^2) (Table III). The total inflammatory burden was the largest in 35-year-olds (13.51 cm^2) and the smallest in 85-year-olds (5.19 cm^2).

Table II. Average values of circumferences of teeth necks for men and women.

		Men			Women	
		Circumference (mm)			Circumference (mm)	
Tooth	*n*	*M*	SD	*n*	*M*	SD
11, 21	15	22.94	0.88	23	22.56	1.26
12, 22	11	20.58	2.07	23	19.69	1.85
13, 23	20	23.91	1.38	12	23.14	1.47
14, 24	34	24.74	1.81	39	23.95	2.02
15, 25	15	23.98	1.59	10	22.44	1.65
16, 26	10	32.67	3.32	10	30.20	2.19
17, 27	15	31.86	2.36	18	30.95	2.71
18, 28	25	30.02	2.67	30	27.68	2.41
31, 41	10	17.53	1.10	18	16.07	1.30
32, 42	12	18.03	1.06	15	16.34	1.71
33, 43	10	21.12	1.62	10	20.30	1.17
34, 44	11	21.46	2.08	12	21.28	2.04
35, 45	17	21.85	1.35	19	21.15	2.16
36, 46	10	32.19	2.47	10	30.21	2.27
37, 47	19	31.80	2.33	10	30.48	2.31
38, 48	10	29.70	2.26	12	28.56	1.27
All	244	808.72*	18.91	271	770.00	18.61

*$p < 0.05$ men vs women.

Table III. The average values of subgingival area, inflammatory burden and periodontal wound size in Ljubljana citizens according to gender and age (n = 238).

			No of teeth		Periodontal wound								Total subgingival area (a + b + c + d)(cm^2)		Total inflammatory burden(b + c + d)(cm^2)		Total bleeding wound (b + d) (cm^2)	
					Healthy subgingival area (a)(cm^2)		Shallow bleeding wound (b) (cm^2)		Deepnon-bleeding area (c)(cm^2)		Deep bleeding wound (d) (cm^2)							
Age	Gender	N	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
35	Men	24	27	1	5.69	2.73	0.62	0.62	9.93	4.53	4.22	3.36	20.48	4.22	14.78	6.40	4.85	3.50
	Women	26	27	1	6.30	2.17	0.47	0.26	9.27	3.57	2.63	3.31	18.69	3.21	12.39	4.83	3.11	3.41
	All	50	27	1	6.10	2.44	0.54	0.47	9.58	4.02	3.38	3.40	19.53	3.78	13.51	5.69	3.93	3.53
45	Men	9	24	2	7.20*	**2.42**	0.66*	**0.33**	6.89	3.64	1.98	1.78	16.75	3.59	9.54	5.03	2.64	1.72
	Women	12	23	6	**4.77**	**2.70**	**0.34**	**0.32**	7.31	4.02	2.79	3.17	15.23	3.71	10.45	3.62	3.14	3.19
	All	21	24	5	5.81	2.81	0.48	0.35	7.13	3.77	2.44	2.64	15.88	3.65	10.06	4.19	2.92	2.62
55	Men	17	17	8	2.59	2.28	0.19*	0.17	6.31	3.89	4.07	3.40	13.17	5.30	10.57	4.40	4.26	3.37
	Women	31	20	6	2.60	2.10	0.44	0.46	8.06	5.28	3.54	2.78	14.66	5.08	12.05	5.19	3.99	2.92
	All	48	19	7	2.60	2.14	0.35	0.40	7.44	4.87	3.73	2.99	14.13	5.16	11.53	4.93	**4.09**	**3.06**
65	Men	22	15	8	2.53*	2.69	0.23	0.22	5.19	3.65	3.21*	4.21	11.17	6.47	8.64	5.76	3.45*	**4.24**
	Women	34	18	8	**4.21**	2.94	0.26	0.33	5.01	2.80	1.23	1.23	10.72	4.84	6.51	3.23	1.49	1.35
	All	56	17	8	3.53	2.94	0.25	0.29	5.08	3.14	2.02	2.96	10.90	5.49	7.36	4.49	2.27	3.00
75	Men	13	14	9	3.47	2.36	0.19	0.14	5.75	3.98	1.02	1.30	10.44	6.18	6.97	4.32	1.21	1.32
	Women	29	13	8	2.88	2.39	0.22	0.31	3.64	3.25	1.32	1.45	8.08	5.21	5.19	3.99	1.55	1.60
	All	42	14	8	3.05	2.37	0.21	0.27	4.26	3.56	1.23	1.39	8.77	5.54	5.71	4.11	1.45	1.52
85	Men	15	9	5	1.35*	**1.29**	0.06*	0.10	3.10	2.34	1.50	2.17	6.03*	4.03	4.67	3.47	1.57	2.19
	Women	6	18	7	**4.32**	**2.29**	**0.20**	0.16	4.65	2.84	1.28	1.39	10.47	4.44	6.14	3.21	1.48	1.50
	All	21	11	7	2.20	2.09	0.10	0.13	3.54	2.52	1.44	1.95	7.30	4.53	5.09	3.39	1.54	1.98
All	Men	100	18	9	3.65	2.99	0.32	0.40	6.40	4.33	2.95*	**3.31**	13.34	7.00	9.69	6.08	3.28*	**3.39**
	Women	138	20	8	4.01	2.76	0.34	0.35	6.40	4.31	**2.18**	**2.51**	12.95	5.87	8.93	5.18	**2.52**	**2.64**
	All	238	19	8	3.86	2.86	0.33	0.37	6.40	4.31	2.50	2.89	13.11	6.35	9.25	5.57	2.84	2.99

*$p < 0.05$ vs. womens of the same age group.

The total bleeding wound was the largest in 55-year-olds (4.09 cm^2) and the smallest in 75-year-olds (1.45 cm^2). Total inflammatory burden was the largest in 35-year-olds (13.51 ± 5.69 cm^2), dropped in 45-year-olds (10.06 ± 4.19 cm^2) and achieved another peak in 55-year olds (11.53 ± 4.93 cm^2). Taken together, the largest total bleeding and deep bleeding wound size was found in 55-year-olds, while the largest total inflammatory burden, the deep non-bleeding area, shallow bleeding wound and healthy subgingival area were found in 35-year-olds (Figure 2). The total average subgingival area in men was found to be slightly larger (13.34 ± 7.00 cm^2) than in women (12.95 ± 5.87 cm^2). The comparison between men and women demonstrated a slightly greater healthy subgingival area and shallow bleeding wound in women and the same average deep non-bleeding area. However, men proved to have a significantly

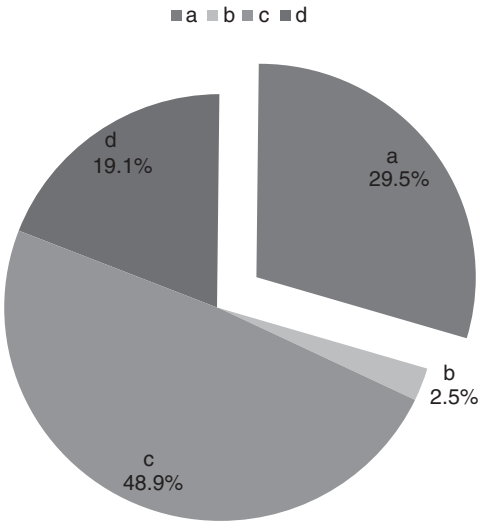

Figure 1. Percentage of healthy subgingival area (a); shallow bleeding wound (b), deep non-bleeding area (c) and deep bleeding wound (d) in 238 citizens of Ljubljana.

larger size of deep bleeding wound (2.95 ± 3.31 cm^2 vs 2.18 ± 2.51 cm^2) ($p < 0.05$), total bleeding wound (3.28 ± 3.39 cm^2 vs 2.52 ± 2.64 cm^2) ($p < 0.05$) and non-significantly larger total inflammatory burden (9.69 ± 6.08 cm^2 vs 8.93 ± 5.18 cm^2).

Measurements of the total inflammatory burden size for 92 subjects in the test group ranged from 1.25–19.23 cm^2, on average 8.96 ± 4.10 cm^2. Men had on average a non-significantly larger inflammatory burden (10.06 ± 4.23 cm^2; range 1.29–19.23 cm^2) than women (8.47 ± 3.84 cm^2; range 1.33–18.26 cm^2).

The results of CRP serum levels demonstrated that values among a total population of 238 subjects ranged from 0.10–32.40 mg/l (average 2.84 ± 3.65 mg/l). Women had non-significantly higher CRP serum levels than men (women: range 0.10–20.05 mg/l, average 2.93 ± 3.28 mg/l; men: range 0.14–32.40 mg/l, average 2.73 ± 4.12 mg/l).

Evaluation of the CRP serum level among 92 subjects in the test group was found to be in the range from 0.10–5.58 mg/l (average 1.40 ± 1.01 mg/l). Also in this group women had non-significantly higher CRP serum levels than men (women: range 0.10–3.57 mg/l, average 1.45 ± 0.97 mg/l; men: range 0.14–5.58 mg/l, average 1.30 ± 1.09 mg/l).

When we correlated the size of the total inflammatory burden and serum levels of CRP in 92 subjects we found a statistically significant correlation for men ($r = 0.49$, $p < 0.05$), women ($r = 0.37$, $p < 0.05$) and total population ($r = 0.37$, $p < 0.05$). The difference between correlation coefficients in men and women was not statistically significant (Figure 3).

Discussion

In the present study we proposed a new method for evaluating the size of the total subgingival area, periodontal bleeding wound and total inflammatory burden based on the measurements of average cervical circumferences of teeth, probing depth and bleeding on probing. We tested the method on a random population of 238 Ljubljana citizens in the age range of 35–85 years. We found that, on average, the subgingival area was 13.11 ± 6.35 cm^2 in size. Out of the total subgingival area, 70.5% (9.25 ± 5.57 cm^2) was represented by the total inflamed and ulcerated subgingival area, which could potentially represent the inflammatory burden of periodontal disease toward the systemic health.

The total inflammatory burden was found to be the largest in the 35-year old population, which could be in correlation with the greatest number of teeth present compared to other age groups (Table III). The

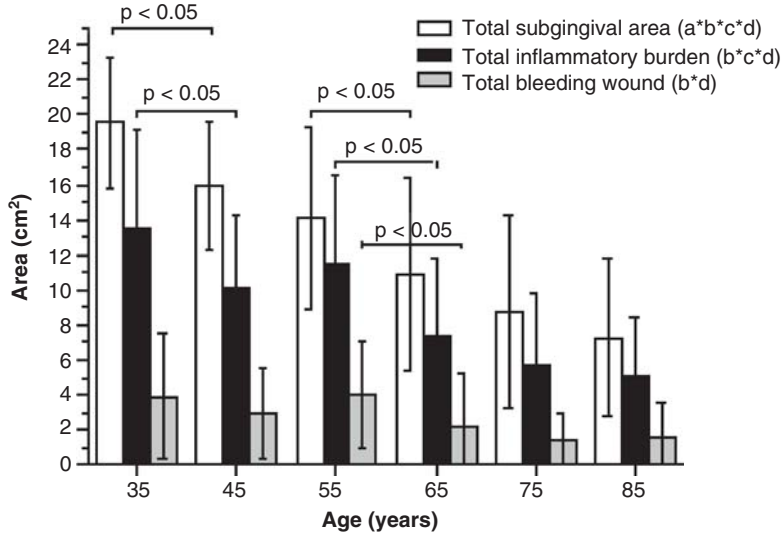

Figure 2. The total subgingival area, total inflammatory burden and total bleeding periodontal wound in cm^2 among 238 citizens of Ljubljana.

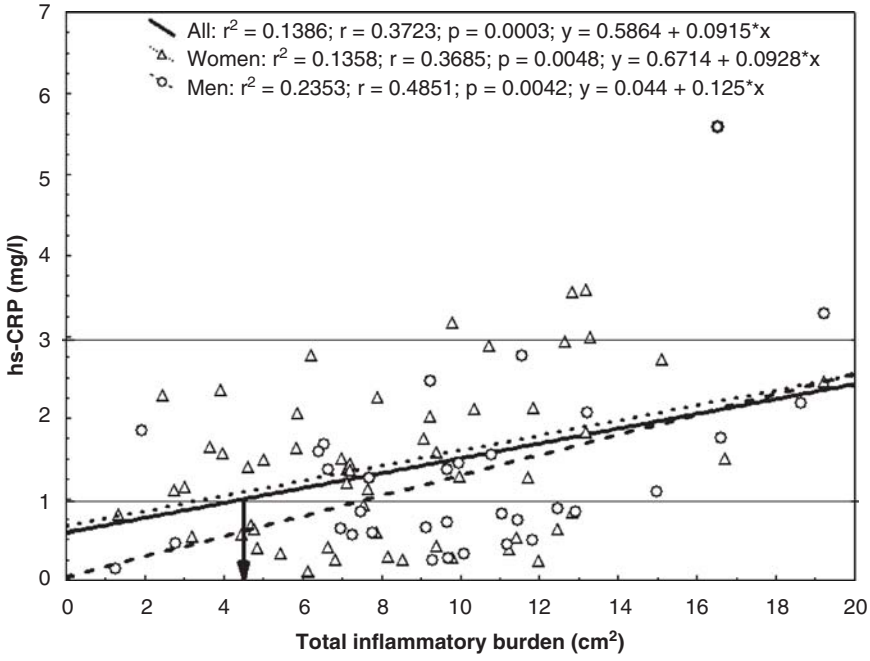

Figure 3. Dose-response relationship between the total inflammatory burden and hsCRP.

larger inflamed subgingival area and periodontal bleeding wound size in men than in women found in our study is in correlation with multiple studies [57–59] demonstrating that the men's periodontal health was worse than that of women. We can assume that a larger inflamed subgingival area and bleeding periodontal wound in men represents a greater inflammatory burden of periodontitis for systemic diseases and conditions. Indeed it was demonstrated [60] that long-term periodontitis in men but not in women is correlated with sub-clinical atherosclerosis.

Our results do not support the results of some studies reporting that the surface area of periodontal pockets is equivalent to that of the palm of the hand (50–75 cm^2) or that of the ventral surface of the forearm ($\cong$ 200 cm^2) [45,46]. These estimates appear inflated since the root surface area of the typical dentition (excluding third molars) is estimated to be ~ 75 cm^2 [61]. Our results are however in accordance with the results of Hujoel et al. [47], who calculated that the typical dentogingival epithelial surface area among individuals with periodontitis ranged from 8–20 cm^2.

More recently, the inflammatory burden of periodontitis was quantified by measuring the periodontal inflamed area. Nesse et al. [44] found that the size of periodontal inflamed surface area ranges from 0.3 cm^2 in healthy individuals to 39 cm^2 in patients with severe generalized periodontitis. In our study we found the total inflammatory burden area ranged from 5.09 ± 3.39 cm^2 in 85-year-old subjects to 13.51 ± 5.69 cm^2 in 35-year-old subjects, on average for all examined subjects 9.25 ± 5.57 cm^2.

Our findings of the positive correlation between the size of the periodontal inflammatory burden and serum levels of hsCRP are in accordance with other studies that found increased levels of hsCRP in patients with localized and generalized aggressive periodontitis [62] where clinical parameters of gingival inflammation, pocket depth and attachment loss were measured and in chronic periodontitis patients [63] where the clinical parameter of radiographic bone loss was used.

However, clinical periodontal disease measurements that were used to quantify the level of periodontal inflammation exposure in these studies provide only a uni-dimensional estimate of the burden of periodontal disease. Our method represents a more quantified assessment of the total periodontal inflammatory burden and also a new two-dimensional approach to study the influence of periodontal inflammatory burden on systemic biomarkers of inflammation such as CRP. According to Ridker [64] the serum levels of CRP below 1 mg/l represent low risk, values between 1–3 mg/l intermediate risk and levels above 3 mg/l a high risk for future cardiovascular events including myocardial infarction and stroke. We can observe in our study (Figure 3) that the inflammatory burden size larger than 4.5 cm^2 represents a moderate risk for cardiovascular disease.

We are aware that our method to quantify the inflammatory burden has limitations for using the parameter of average length of the neck circumferences of the individual teeth in men and women. The gingival margin can be below or above the cemento-enamel junction and so the inclusion of the length of the tooth neck circumference in the formula could be

over-estimated in the case of gingival recession and under-estimated in the case of pseudopockets or gingival over-growth.

However, our improved two-dimensional method for evaluation of inflammatory burden is new and should be tested on patients with periodontal disease and systemic diseases or conditions such as cardiovascular disease, diabetes and pregnancy.

Recently, the first study [65] was published demonstrating a dose–response relationship between periodontal inflamed surface area and HbA1c in type 2 diabetes patients. Interventional periodontal treatment studies are needed to demonstrate that reduced size of inflammatory burden correlates with lower levels of markers of systemic inflammation such as CRP.

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References

[1] Hugoson A, Norderyd O. Has the prevalence of periodontitis changed during the last 30 years? J Clin Periodontol 2008;35 (Suppl 8):338–45.

[2] Al-Shammari KF, Al-Khabbaz AK, Al-Ansari JM, Neiva R, Wang HL. Risk indicators for tooth loss due to periodontal disease. J Periodontol 2005;76:1910–8.

[3] Burt B. Position paper: epidemiology of periodontal diseases. J Periodontol 2005;76:1406–19.

[4] Bahekar AA, Singh S, Saha S, Molnar J, Arora R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. Am Heart J 2007;154:830–7.

[5] Khader YS, Albashaireh ZS, Alomari MA. Periodontal diseases and the risk of coronary heart and cerebrovascular diseases: a meta-analysis. J Periodontol 2004;75: 1046–53.

[6] Janket SJ, Baird AE, Chuang SK, Jones JA. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:559–69.

[7] Saremi A, Nelson RG, Tulloch-Reid M, Hanson RL, Sievers ML, Taylor GW, et al. Periodontal disease and mortality in type 2 diabetes. Diabetes Care 2005;28:27–32.

[8] Khader YS, Dauod AS, El-Qaderi SS, Alkafajei A, Batayha WQ. Periodontal status of diabetics compared with nondiabetics: a meta-analysis. J Diabetes Complications 2006; 20:59–68.

[9] Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for nosocomial bacterial pneumonia and chronic obstructive pulmonary disease. A systematic review. Ann Periodontol 2003;8:54–69.

[10] Khader YS, Ta'ani Q. Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis. J Periodontol 2005;76:161–5.

[11] Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, et al. Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol 1996;67(Suppl 10): 1103–13.

[12] Scannapieco FA, Bush RB, Paju S. Periodontal disease as a risk factor for adverse pregnancy outcomes. A systematic review. Ann Periodontol 2003;8:70–8.

[13] Wimmer G, Pihlstrom BL. A critical assessment of adverse pregnancy outcome and periodontal disease. J Clin Periodontol 2008;35(Suppl 8):380–97.

[14] Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008;35:277–90.

[15] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805–12.

[16] Dhingra R, Gona P, Nam BH, D'Agostino RB, Sr, Wilson PW, Benjamin EJ, et al. C-reactive protein, inflammatory conditions, and cardiovascular disease risk. Am J Med 2007;120:1054–62.

[17] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998;279:1477–82.

[18] Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation 2004;109(25 Suppl 1):IV6–19; doi: 10.1161/01.CIR.0000133444.17867.56.

[19] Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.

[20] Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010; 375:132–40.

[21] Moshage HJ, Roelofs HM, van Pelt JF, Hazenberg BP, van Leeuwen MA, Limburg PC, et al. The effect of interleukin-1, interleukin-6 and its interrelationship on the synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes. Biochem Biophys Res Commun 1988;155:112–7.

[22] Inoue N. Vascular C-reactive protein in the pathogenesis of coronary artery disease: role of vascular inflammation and oxidative stress. Cardiovasc Hematol Disord Drug Targets 2006;6:227–31.

[23] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.

[24] Maekawa T, Tabeta K, Kajita-Okui K, Nakajima T, Yamazaki K. Increased expression of C-reactive protein gene in inflamed gingival tissues could be derived from endothelial cells stimulated with interleukin-6. Arch Oral Biol 2011;56:1312–8.

[25] Wolbink GJ, Brouwer MC, Buysmann S, ten Berge IJ, Hack CE. CRP-mediated activation of complement in vivo: assessment by measuring circulating complement-C-reactive protein complexes. J Immunol 1996;157:473–9.

[26] Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102:2165–8.

[27] Pasceri V, Cheng JS, Willerson JT, Yeh ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by antiatherosclerosis drugs. Circulation 2001;103:2531–4.

[28] Montero I, Orbe J, Varo N, Beloqui O, Monreal JI, Rodriguez JA, et al. C-reactive protein induces matrix

metalloproteinase-1 and -10 in human endothelial cells: implications for clinical and subclinical atherosclerosis. J Am Coll Cardiol 2006;47:1369–78.
[29] Jandik KA, Belanger M, Low SL, Dorn BR, Yang MC, Progulske-Fox A. Invasive differences among Porphyromonas gingivalis strains from healthy and diseased periodontal sites. J Periodontal Res 2008;43:524–30.
[30] Rautemaa R, Jarvensivu A, Kari K, Wahlgren J, DeCarlo A, Richardson M, et al. Intracellular localization of Porphyromonas gingivalis thiol proteinase in periodontal tissues of chronic periodontitis patients. Oral Dis 2004;10:298–305.
[31] Nishimura F, Taniguchi A, Iwamoto Y, Soga Y, Fukushima M, Nagasaka S, et al. Porphyromonas gingivalis infection is associated with elevated C-reactive protein in nonobese Japanese type 2 diabetic subjects. Diabetes Care 2002;25:1888.
[32] Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol 2001;72: 1221–7.
[33] Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. J Dent Res 2000;79:49–57.
[34] Yamaguchi M, Nishimura F, Naruishi H, Soga Y, Kokeguchi S, Takashiba S. Thiazolidinedione (pioglitazone) blocks P. gingivalis- and F. nucleatum, but not E. coli, lipopolysaccharide (LPS)-induced interleukin-6 (IL-6) production in adipocytes. J Dent Res 2005;84:240–4.
[35] Okabe K, Nakagawa K, Yamamoto E. Factors affecting the occurrence of bacteremia associated with tooth extraction. Int J Oral Maxillofac Surg 1995;24:239–42.
[36] Debelian GJ, Olsen I, Tronstad L. Bacteremia in conjunction with endodontic therapy. Endod Dent Traumatol 1995;11: 142–9.
[37] Lafaurie GI, Mayorga-Fayad I, Torres MF, Castillo DM, Aya MR, Baron A, et al. Periodontopathic microorganisms in peripheric blood after scaling and root planing. J Clin Periodontol 2007;34:873–9.
[38] Lucartorto FM, Franker CK, Maza J. Postscaling bacteremia in HIV-associated gingivitis and periodontitis. Oral Surg Oral Med Oral Pathol 1992;73:550–4.
[39] Lofthus JE, Waki MY, Jolkovsky DL, Otomo-Corgel J, Newman MG, Flemmig T, et al. Bacteremia following subgingival irrigation and scaling and root planing. J Periodontol 1991;62:602–7.
[40] Silver JG, Martin AW, McBride BC. Experimental transient bacteraemias in human subjects with varying degrees of plaque accumulation and gingival inflammation. J Clin Periodontol 1977;4:92–9.
[41] Geerts SO, Nys M, De MP, Charpentier J, Albert A, Legrand V, et al. Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. J Periodontol 2002;73:73–8.
[42] Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 2002;252:283–94.
[43] Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. J Periodontol 1996;67(Suppl 10):1123–37.
[44] Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. J Clin Periodontol 2008;35: 668–73.
[45] Offenbacher S, Beck JD, Lieff S, Slade G. Role of periodontitis in systemic health: spontaneous preterm birth. J Dent Educ 1998;62:852–8.
[46] Page RC. The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. Ann Periodontol 1998;3:108–20.
[47] Hujoel PP, White BA, Garcia RI, Listgarten MA. The dentogingival epithelial surface area revisited. J Periodontal Res 2001;36:48–55.
[48] Kovac-Kavcic M, Skaleric U. The change of periodontal treatment needs in a population of Ljubljana, Slovenia, over a ten year period. J Int Acad Periodontol 2000;2:94–100.
[49] Skaleric U, Kovac-Kavcic M. Periodontal treatment needs in a population of Ljubljana, Yugoslavia. Community Dent Oral Epidemiol 1989;17:304–6.
[50] Skaleric E, Petelin M, Kovac-Kavcic M, Skaleric U. Periodontal treatment needs in population of Ljubljana - twenty years after first examination. Slovenian Dental Journal 2008; 63:63–6.
[51] King DE, Mainous AG, 3rd, Buchanan TA, Pearson WS. C-reactive protein and glycemic control in adults with diabetes. Diabetes Care 2003;26:1535–9.
[52] Zhang HF, Zhu ZY, Yao WM, Xu F, Liang NX, Gong L, et al. [Relationship between high-sensitive c-reactive and blood pressure]. Zhonghua Xin Xue Guan Bing Za Zhi 2010;38:993–7.
[53] Sennels H, Sorensen S, Ostergaard M, Knudsen L, Hansen M, Skjodt H, et al. Circulating levels of osteopontin, osteoprotegerin, total soluble receptor activator of nuclear factor-kappa B ligand, and high-sensitivity C-reactive protein in patients with active rheumatoid arthritis randomized to etanercept alone or in combination with methotrexate. Scand J Rheumatol 2008;37:241–7.
[54] Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk in Communities study. Arch Intern Med 2003;163: 1172–9.
[55] Rooks C, Faber T, Votaw J, Veledar E, Goldberg J, Raggi P, et al. Effects of smoking on coronary microcirculatory function: a twin study. Atherosclerosis 2011;215:500–6.
[56] Kones R. Rosuvastatin, inflammation, C-reactive protein, JUPITER, and primary prevention of cardiovascular disease–a perspective. Drug Des Devel Ther 2010;4:383–413.
[57] Hugoson A, Laurell L, Lundgren D. Frequency distribution of individuals aged 20–70 years according to severity of periodontal disease experience in 1973 and 1983. J Clin Periodontol 1992;19:227–32.
[58] Brown LJ, Oliver RC, Loe H. Periodontal diseases in the U.S. in 1981: prevalence, severity, extent, and role in tooth mortality. J Periodontol 1989;60:363–70.
[59] Albandar JM. Global risk factors and risk indicators for periodontal diseases. Periodontol 2000 2002;29:177–206.
[60] Desvarieux M, Schwahn C, Volzke H, Demmer RT, Ludemann J, Kessler C, et al. Gender differences in the relationship between periodontal disease, tooth loss, and atherosclerosis. Stroke 2004;35:2029–35.
[61] Hujoel PP. A meta-analysis of normal ranges for root surface areas of the permanent dentition. J Clin Periodontol 1994;21: 225–9.
[62] Salzberg TN, Overstreet BT, Rogers JD, Califano JV, Best AM, Schenkein HA. C-reactive protein levels in patients with aggressive periodontitis. J Periodontol 2006;77:933–9.
[63] Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol 2000;71:1528–34.
[64] Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363–9.
[65] Nesse W, Linde A, Abbas F, Spijkervet FK, Dijkstra PU, de Brabander EC, et al. Dose-response relationship between periodontal inflamed surface area and HbA1c in type 2 diabetics. J Clin Periodontol 2009;36:295–300.