Clin Oral Invest (2012) 16:1199–1207
DOI 10.1007/s00784-011-0627-7

ORIGINAL ARTICLE

Increased systemic elastase and C-reactive protein in aggressive periodontitis (CLOI-D-00160R2)

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Received: 11 March 2011 / Accepted: 5 October 2011 / Published online: 19 October 2011

Abstract The inflammatory mediators, serum elastase and C-reactive protein (CRP), are associated with an increased risk for coronary heart disease. Thus, the aim of this study is to compare systemic inflammatory mediators in periodontally healthy controls (C), patients with untreated aggressive (AgP) and chronic (ChP) periodontitis. C [periodontal pocket probing depth (PPD) <3.6 or <5 mm without bleeding (BOP), BOP<10%], ChP (PDD≥3.6 mm and probing attachment loss ≥5 mm at >30% of sites; age >35 years), and AgP (clinically healthy; PDD≥3.6 mm at >30% of sites, bone loss ≥50% at ≥2 teeth; age ≤35 years) were examined clinically, and the body mass index was assessed. Blood was sampled for assessment of serum levels of elastase, CRP, lipopolysaccharide binding protein (LBP), interleukin (IL) 6, 8, and leukocyte counts. Thirty C, 31 ChP, and 29 AgP were analyzed. Elastase, CRP, LBP, and IL-6 levels were elevated in AgP compared to C (p<0.013), whereas leukocyte counts and IL-8 were similar. Multiple regression analysis identified AgP (p<0.001) and education level (p<0.001) to explain 47% of the variation of elastase. AgP (p=0.003), African origin (p=0.006), female sex (p=0.002), and BMI (p<0.001) explained 39% of the variation of CRP. Serum elastase and CRP are significantly elevated in AgP compared to C. AgP patients exhibit a stronger systemic inflammatory burden than C patients.

Keywords C-reactive protein · Leukocyte elastase · Lipopolysaccharide-binding protein · Interleukin-6 · Aggressive periodontitis · Chronic periodontitis

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Introduction

Periodontitis, the infectious-inflammatory destruction of tooth supporting tissues (i.e., connective tissue and bone), is a widespread disease throughout the world [1]. Untreated periodontitis is characterized by periodontal pockets and loss of tooth supporting tissues (attachment loss). Patients with periodontal disease experience bacteremia after tooth brushing, flossing, and chewing [2]. The host responds to these constantly repeated bacteremia and systemic spread of proinflammatory cytokines from periodontal pockets in a similar manner as in other cases of chronic infections or inflammatory processes.

There is strong evidence from cross-sectional studies that serum CRP in periodontitis patients is elevated compared to healthy controls [3–5]. Patients with cardiovascular disease were found to exhibit more severe periodontitis than cardiovascularly healthy controls [6]. Regarding serum CRP, the American Heart Association distinguishes between three concentration intervals with increasing risk for

coronary heart disease (CHD): low, CRP<0.1 mg/dl; moderate, CRP=0.1–0.3 mg/dl; high, CRP>0.3 mg/dl [7]. The increase in serum CRP caused by periodontitis may be one link connecting or explaining the correlation between periodontal and cardiovascular disease [6–13]. However, there is no information whether different types of periodontitis (chronic: ChP/aggressive: AgP) cause different levels of systemic inflammation. Serum neutrophil elastase is another systemic inflammatory parameter. Serum elastase levels are increased in chronic infections such as chronic obstructive pulmonary disease [14]. Serum neutrophil elastase is elevated in obese prehypertensive women and associated with airflow dysfunction [15]. Furthermore, serum neutrophil elastase activity is increased in isolated systolic hypertension and is associated to pulse wave velocity [16]. Thus, increased serum neutrophil elastase is considered a risk factor for cardiovascular and respiratory disease. Lipopolysaccharide-binding protein (LBP) is a glycosylated protein that is synthesized by hepatocytes. It is released into the blood through acute phase stimulation and has been shown to be increased in AgP [17].

The aim of this study therefore was to compare inflammatory serum parameters in periodontally healthy controls and patients with untreated AgP as well as generalized severe ChP.

Materials and methods

Forty periodontally healthy controls and 66 patients with untreated severe periodontal disease (34 with generalized severe ChP and 32 with AgP) were recruited at the Department of Periodontology of the Centre for Dental, Oral, and Maxillofacial Medicine (Carolinum), Johann Wolfgang Goethe-University Frankfurt/Main. Controls and patients had to fulfill the following criteria:

Inclusion criteria:

- At least 16 years of age
- At least 20 remaining teeth
- Written informed consent

Healthy controls (C; control)

- Probing pocket depths (PPD) <3.6 mm and from 3.6 to 4 mm without bleeding on probing (BOP), up to one site with a PPD from 3.6 to 5 mm without BOP was accepted
- BOP <10%

Aggressive periodontitis (AgP; case 1)

- Patient is clinically healthy, i.e., he or she does not suffer from systemic diseases predisposing himself/herself to periodontitis (e.g., diabetes mellitus)
- Probing pocket depths (PPD) ≥3.6 mm at >30% of sites
- Radiographic bone loss ≥50% at a minimum of two separate teeth
- Age at time of diagnosis ≤35 years
- ≤37 years of age

Generalized severe chronic periodontitis (ChP; case 2)

- PPD≥3.6 mm and vertical clinical attachment loss (PAL-V) ≥5 mm at more than 30% of sites
- PPD≥7 mm at a minimum of four sites
- >35 years of age

Exclusion criteria:

- Requirement of systemic antibiotics for measures that may cause transitory bacteremia (e.g., pocket probing)
- Self-reported chronic disease influencing the serum CRP level (e.g., rheumatoid arthritis, Crohn's disease or ulcerative colitis)
- Self-reported infectious disease within the last 8 weeks before examination (history of fever)
- Nonsurgical or surgical periodontal treatment within the last 24 months before examination
- Systemic or topical subgingival antibiotics within the last 8 weeks before examination

All controls and patients were asked about their actual body weight and height. Furthermore, all were asked about current and past cigarette smoking habit. Patients who reported to smoke or had quit smoking for <5 years were classified as smokers [18]. Additionally ethnical origin (country, place of birth of individual, as well as her or his parents) and level of education [general education secondary school (Hauptschule), junior high school (Realschule), high school (Gymnasium)] as surrogate for socioeconomic status were recorded.

The study complied with the rules of the Declaration of Helsinki and was approved by the Institutional Review Board for Human Studies of the Medical Faculty of the Johann Wolfgang Goethe-University Frankfurt/Main (application number 188/06). All participating individuals were informed on risks and benefit as well as the procedures of the study and gave written informed consent.

Clinical examination

The following clinical parameters were assessed at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual):

- Modified gingival bleeding index (GBI) at six sites per tooth [19]
- Modified plaque control record (PCR) at six sites per tooth [20]
- PPD to the nearest 0.2 mm using an electronic probe (Florida Probe, Version 3.2, Gainesville, FL, USA)

- BOP 30 s after probing
- Recession to the nearest 0.5 mm using a manual periodontal probe (PCPUNC 15, HuFriedy, Chicago, IL, USA) from the cemento-enamel junction (CEJ) to the gingival margin. At sites where the CEJ was destroyed by restorations, the restoration margin (RM) was used as reference. At sites where the CEJ or RM was located apically from the gingival margin, the value for recession was negative.

Attachment loss was calculated as sum of PPD and recession.

In seven patients, PPD measurements in one quadrant were repeated.

Blood samples

Immediately prior to the clinical examination, 20 ml of blood was sampled from an arm vein. Physical activity prior to sampling was excluded. Intake of food was not standardized.

Serum levels of CRP, elastase, and leucocytes count were analyzed at the Department of Laboratory Medicine of the Centre for Internal Medicine, Hospital of the Johann Wolfgang Goethe-University Frankfurt/Main. Serum CRP levels were assessed using an immunoturbidimetric assay (CRPLX, Cobas, Roche Diagnostics GmbH, Mannheim, Germany) for the in vitro quantitative determination with the Roche/Hitachi 917 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The detection limit for CRP was 0.01 mg/dl. Serum PMN elastase was assessed with an enzyme-linked immunosorbent assay (ELISA; human PMN elastase BMS269CE, Bender MedSystems GmbH, Vienna, Austria) using a SLT-Spectra photometer (SLT Labinstruments, Crailsheim, Germany). Absorbance was measured at 450 nm. The detection limit for elastase was 1.98 ng/ml. Leukocyte counts were determined according to the electrical resistance measurement principle: An amount of the sample was aspirated and diluted in a conductive fluid. The difference between diluted fluid and blood cells regarding conductivity was used to count them (Sysmex SE-9500; Sysmex Deutschland GmbH, Norderstedt, Germany).

At the Children's Hospital, Pneumology, Allergology and Mucoviscidosis, Hospital of the Johann Wolfgang Goethe-University Frankfurt/Main IL-6, IL-8, and LBP serum concentrations were measured in duplicates by ELISA technique according to manufacturers' instructions. The assay sensitivity for IL-6 (R&D Systems, Minneapolis, MN, USA) was <0.7 pg/ml, the detection limit for IL-8 was <10.0 pg/ml (Becton Dickinson, San Jose, CA, USA), and measurable concentration range of LBP was between 0.5 and 50 ng/ml (Biometec, Greifswald, Germany).

Statistical analysis

Serum elastase and CRP were defined as main outcome variables. Secondary outcome variables were leukocyte counts, LBP, IL-6, and IL-8. To find a difference of 0.22 mg/dl of serum CRP between two groups with a type 1 error $\alpha<0.05$ and a test power of 80% for standard deviations of 0.3 mg/dl a minimal sample size $n=30$ per group is required [21].

Except for leukocyte counts, serum inflammatory parameters were not normally distributed and were log-transformed for statistical analysis [21–23].

Repeated PPD measurements in one quadrant of each of seven patients were used to estimate the reproducibility of PPD measurements as percentage of repeated measurements with a difference ≤1 mm and standard deviation of single measurements [24].

For all individuals the body mass index (BMI) and cigarette pack years were calculated. Dichotomous control/patient characteristics (sex, current smoking) were expressed as frequencies for each group separately (C, ChP, and AgP). For all other control/patient characteristics (age, number of remaining teeth, pack years, BMI, GBI, PCR, and BOP), means±standard deviations were calculated for each group separately. For all site-based periodontal parameters (PPD and PAL-V), means per individual were calculated from which means±standard deviations were calculated for each group separately. Furthermore, the sum of all PPD was calculated per individual to describe the size of the interface between periodontal pocket and vascular system [22]. Comparisons between groups for dichotomous parameters were made by χ^2 test, for serum parameters by analysis of covariance adjusting for age, level of education, pack years, BMI, and African origin. Adjusting for multiple testing with the main outcome variables, the level of significance was set to $p<0.013$. For all other parameters, comparisons were made by analysis of variance with post hoc test and Bonferroni adjustment for multiple testing.

Using stepwise linear backward multiple regression analysis, factors should be identified that influenced the dependent variables serum elastase, CRP, leukocyte counts, serum LBP, IL-6, and IL-8. The following independent variables were entered into the models: diagnosis, sex, age, ethnical origin (European, Asian, and African), BMI, PCR, BOP, smoking (current/never and former), pack years, mean PPD per individual, and sum of all PPD per individual. The following parameters were described by dummy variables: diagnosis (C/ChP/AgP=0:1:0, C/ChP/AgP=0:0:1), sex (male=0, female=1), level of education (general education secondary school/junior high school/high school=0:1:0; general education secondary school/junior high school/high school=0:0:1), ethnical (European/Asian/African=0:1:0; European/Asian/African=0:0:1),

smoking status (never and former smoker=0; current smoker=1). GBI was not entered into analysis because of collinearity to BOP. All factors with $p<0.05$ were kept in the models. For statistical analysis, a PC program was used (Systat™ for Windows Version 10, Systat Inc. Evanston, IL USA).

Results

Thirty periodontally healthy controls, 31 ChP, and 29 AgP patients were included between October 2006 and December 2009. Ten controls [22] and three patients were recruited but not included because they did not meet the inclusion criteria. Three patients did not show up for examination. It was more difficult to find and took more time to recruit AgP patients fulfilling the inclusion criteria than expected. In 2009, it was decided to finish recruitment at the end of year 2009; therefore; only 29 AgP patients could be included and not the anticipated 30. All controls but one (Korean) were of European descent (22 German, three Turkish, one Spanish, one French, one Croatian, and one mixed German/Spanish). All controls were dental students or faculty members. All ChP patients but two (one Japanese, one Sri Lankan) were also of European origin (23 German, two Greek, two Turkish, one mixed French/Portuguese, and one mixed German/Portuguese). The AgP group was ethnically more heterogeneous: 20 Europeans (13 German, three Italian, one Greek, two mixed Romanian/Hungarian, and one mixed Italian/Greek), five Asian (two Pakistani, one Sri Lankan, one Chinese, and one Kazakh), and four African (two Moroccan, one Eritrean, and one Togolese). Control/patient parameters are given in Table 1.

To assess reproducibility of PPD measurements, 306 sites in seven patients were measured in duplicate. The difference between both measurements was 1 mm or below in 99% of cases. Standard deviation of single measurements was 0.32 mm. Periodontal parameters are given in Table 2.

Main outcome variables (serum elastase and CRP) are given in Table 3. After adjustment for age, level of education, pack years, BMI, and African origin, elastase and CRP were higher in AgP than in controls. Frequency of individuals with serum CRP levels between 0.1 and 0.3 mg/dl (moderate risk for CHD) was higher in ChP than in controls as well as in AgP than in controls and ChP. Regarding serum CRP levels >0.3 mg/dl (high risk for CHD) frequency of individuals with serum CRP levels >0.3 mg/dl was higher in AgP than in controls and ChP (Table 3). The secondary outcome variables LBP and IL-6 levels were higher in AgP than in controls, too (Table 4).

Backward stepwise linear multiple regression analysis identified AgP ($p<0.001$) to be positively and high school level of education to be negatively associated with serum elastase. The model explained 47% of the variation of the variable (Table 5). CRP was also positively correlated to AgP ($p=0.003$), African origin ($p=0.006$), female sex ($p=0.002$), and BMI ($p<0.001$) with the model explaining 39% of the variation of the variable (Table 6). LBP correlated positively with AgP ($p=0.003$) and negatively with high school level of education ($p=0.047$; Table 7). There was a strong and statistically significant correlation between IL-6 and CRP (Pearson's $R^2=0.58$, $p<0.001$). However, the stepwise regression model for IL-6 revealed AgP ($p<0.001$), ChP ($p<0.001$), and BMI ($p=0.004$) as significant parameters (Table 8).

Discussion

As in cases of other chronic infections or inflammatory processes, the host responds to bacteremia and systemic

Table 1 Individuals' characteristics

Parameters	Healthy controls (C) ($n=30$)	Chronic periodontitis (ChP) ($n=31$)	Aggressive periodontitis (AgP) ($n=29$)	C/ChP/AgP p	Post hoc tests		
					C/ChP p	C/AgP p	ChP/AgP p
Female sex (n)	16/53%	12/39%	16/55%	0.371[a]			
Age (years)	27.5±3.3	52.8±7.6	31.1±5.7	<0.001[b]	<0.001	0.058	<0.001
General education secondary school (n)	0	9/29%	6/21%	0.008[a]	0.001	0.009	0.456
Junior high school (n)	0	9/29%	10/34%	0.002[a]	0.001	<0.001	0.650
High school (n)	30/ 100%	13/42%	13/45%	<0.001[a]	<0.001	<0.001	0.821
Remaining teeth (n)	28.5±1.7	25.9±2.8	28.5±2.1	<0.001[b]	<0.001	1.000	<0.001
Current smokers (n)	8/27%	10/32%	9/31%	0.883[a]			
Pack years (n)	0.8±1.8	11.4±17.9	3.5±5.6	0.025[b]	0.001	1.000	0.021
Body mass index (kg/m^2)	21.8±2.5	25.5±3.8	26.6±5.3	<0.001[b]	0.001	<0.001	0.948

[a] χ^2

[b] Analysis of variance

Table 2 Individuals' periodontal parameters

Parameters	Healthy controls (C) (n=30)	Chronic periodontitis (ChP) (n=31)	Aggressive periodontitis (AgP) (n=29)	ANOVA C/ ChP/ AgP p	Post hoc tests		
					C/ChP p	C/AgP p	ChP/AgP p
Gingival bleeding index (%)	2.6±1.5	15.5±12.2	13.4±9.2	<0.001	<0.001	<0.001	1.000
Plaque control record (%)	14.1±7.7	34.6±17.0	40.2±14.7	<0.001	<0.001	<0.001	0.372
Bleeding on probing (%)	6.8±1.8	55.9±14.4	48.6±13.3	<0.001	<0.001	<0.001	0.043
Probing pocket depth (PPD) (mm)	2.0±0.2	3.9±0.6	3.5±0.7	<0.001	<0.001	<0.001	0.008
Attachment level (mm)	0.4±0.2	4.2±1.7	2.8±1.4	<0.001	<0.001	<0.001	<0.001
Sum of all PPD per person (mm)	324.6±32.3	608.7±113.2	595.8±120.2	<0.001	<0.001	<0.001	1.000

ANOVA analysis of variance

spread of proinflammatory cytokines from periodontal pockets. The production of IL-6 is induced and mediates the liver to produce CRP and other acute-phase proteins [25, 26]. This mechanism was the reason to follow serum IL-6 as a secondary outcome in this study. Bacteremia after periodontal probing is observed more frequently in periodontitis than in gingivitis [27]. The parakeratinized and ulcerated pocket epithelium of established gingivitis and periodontitis forms an easy port of entry for oral microorganisms. If the pocket walls of all periodontally compromised teeth in an untreated patient are combined, the wound surface due to periodontitis is estimated to be as large as 8–20 cm^2 [3]. The size of the wound surface depends primarily on periodontal pocket depths and not attachment loss. After periodontal treatment, periodontal pockets may be resolved while attachment loss will persist. A correlation of serum elastase and CRP with mean PPD was found in individuals with a quite low mean attachment loss of 0.4 mm [2].

Elevated levels of serum elastase [14–16] and CRP [6, 7, 12, 13] are linked to a higher risk for other diseases (e.g., CVD, COPD). Thus, we were particularly interested in these factors and defined them as primary outcomes.

The present study confirms the strong evidence from cross-sectional studies that serum CRP in periodontitis patients is elevated compared to healthy controls [3, 5, 28]. Only one study compared healthy controls, localized ChP, and generalized AgP [29]. Two further studies compared non-periodontitis controls to localized and generalized AgP patients. Both studies found significantly elevated CRP levels in generalized AgP compared to non-periodontitis controls [21, 30]. To the best of our knowledge, up to now, this is the first study comparing healthy controls with generalized ChP and AgP. However, quite recently serum CRP and various cytokines were compared between two groups of patients suffering from generalized severe periodontitis [≥30% of sites with clinical attachment loss and bone loss of one third of the root (≥ 30% of teeth)] and aged 18–40 years. The diagnosis AgP was assigned to individuals (1) exhibiting bone loss ≥3 mm within 3 years as evidenced from consecutive radiographs and (2) familial aggregation established from self-report or, if possible, first degree relatives. Twenty-one individuals were diagnosed ChP and 24 AgP. Although CRP was 0.8 mg/l higher in AgP than in ChP, the study failed to show statistically significant differences between ChP and AgP regarding CRP or other serum parameters [31]. From our point of view, the definition of AgP by Cairo et al. [31] is erroneous. Individuals below the age 35 who already suffer from ≥50%

Table 3 Individuals' serum elastase and C-reactive protein (CRP) levels (main outcome parameters)

Parameters	Healthy controls (C) (n=30)	Chronic periodontitis (ChP) (n=31)	Aggressive periodontitis (AgP) (n=29)	C/ChP/AgP p	Post hoc tests		
					C/ChP p	C/AgP p	ChP/AgP p
Elastase (ng/ml)	9.99±4.73	17.12±12.35	32.00±14.63	<0.001[a]	0.858	<0.001	0.021
CRP[a] (mg/dl)	0.10±0.12	0.17±0.23	0.55±0.98	0.001[a]	0.410	0.003	0.093
CRP[a] 0.1–0.3 mg/dl [n (%)]	6 (20)	19 (61)	27 (93)	<0.001[a]	0.003	<0.001	0.012
CRP[a] >0.3 mg/dl [n (%)]	3 (10)	3 (10)	13 (33)	0.001[a]	1.000	0.009	0.009

[a] Analysis of covariance adjusting for age, level of education, pack years, BMI, and African origin

[b] χ^2

Table 4 Individuals' leukocyte count and serum LPS-binding protein, and interleukin 6 and 8 (secondary outcome variables)

Parameters	Healthy controls (C) (n=30)	Chronic periodontitis (ChP) (n=31)	Aggressive periodontitis (AgP) (n=29)	C/ChP/AgP p	Post hoc tests		
					C/ChP p	C/AgP p	ChP/AgP p
Leukocyte count (nl^{-1})	5.95±1.27	6.29±1.82	6.26±2.48	0.744[b]			
LPS-binding protein[a] (μg/ml)	22.37±11.40	26.87±15.25	35.78±14.60	0.048[b]	0.749	0.004	0.125
Interleukin 6 (pg/ml)	0.69±0.47	1.93±1.16	2.38±3.04	0.026[b]	0.421	0.006	0.608
Interleukin 8 (pg/ml)	20.87±19.76	31.84±35.14	23.45±27.32	0.434[b]			

[a] Lipopolysaccharide

[b] Analysis of covariance adjusting for age, level of education, pack years, BMI, and African origin

bone loss in at least two different sites have experienced rapid destruction. Thus, most patients diagnosed ChP by Cairo et al. [31] would have been AgP based on our definition. Another reason for not finding differences between both groups may be a too small sample.

The case definition for AgP in a clinical trial is difficult. The workshop for the Development of a Classification System for Periodontal Diseases and Conditions of 1999 abandoned age of onset as decisive criterion and defined the following common features of AgP: (1) except for the presence of periodontitis, patients are otherwise clinically healthy; (2) rapid attachment loss and bone destruction; and (3) familial aggregation [32]. Medical history provides whether patients are clinically healthy or not. This criterion is considered as it is in most other studies on AgP. However, what is rapid attachment loss and bone destruction? Localized AgP has its onset around puberty [32]. If periodontitis has caused bone loss of at least 50% before the age of 35, we may judge this as rapid destruction, i.e., 50% bone loss in a timeframe of <25 years. Bone loss ≥3 mm within 3 years as evidenced from consecutive radiographs actually is more rapid [31]. What is rapid enough to be called AgP? How can rapid destruction be assessed in older patients? In a 50-year-old patient, bone loss of 50% may have started at age 15 and developed over 35 years or started at age 35 and developed within 15 years. In most cases, we do not have PAL-V measurements or radiographs to assess when destruction started. Thus, we can only be sure about rapid destruction in young patients. Even the definitions of the 1999 workshop are not consistent, e.g., definitions for localised and generalised AgP by Armitage 1999 (localised, ≤30% of sites/generalised, >30% of sites) and Lang et al. [32] (localised: incisors, first molars, and ≤2 other teeth affected; generalised: incisors, first molars, and >2 other teeth affected) are different. Armitage [33] writes, "It would have been possible to include in the new classification additional subcategories such as "diabetes mellitus-associated aggressive periodontitis" and "diabetes mellitus-associated chronic periodontitis", whereas Lang et al. [32] state that "except for the presence of periodontitis, patients are otherwise clinically healthy." A patient suffering from diabetes mellitus is not clinically healthy. Thus, according to Lang et al. [32], there is no option for subcategories such as "diabetes mellitus-associated aggressive periodontitis." Due to these obvious reasons and inconsistencies of the 1999 workshop, many studies on AgP still use age as a criterion [21, 30, 34].

The groups are well balanced for sex and smoking status. In the multiple regression models serum CRP was higher in women than in men (Tab. 6). Higher CRP levels

Table 5 Backward stepwise multiple regression analysis: serum elastase in relation to individual and periodontal parameters

Dependent variable: log-transformed serum elastase (n=90)				
R^2=0.481; $R^2_{adjusted}$=0.469; standard error of estimate=0.511				
	b	SE (b)	T	p value
Constant	2.736	0.104	26.305	<0.001
Aggressive periodontitis	0.820	0.119	6.889	<0.001
High school	−0.444	0.115	−3.868	<0.001

Analysis of variance: p<0.001

Table 6 Backward stepwise multiple regression analysis: serum C-reactive protein (CRP) in relation to individual and periodontal parameters

Dependent variable: log-transformed serum CRP (n=90)				
R^2=0.420; $R^2_{adjusted}$=0.392; standard error of estimate=0.400				
	b	SE (b)	T	p value
Constant	−2.225	0.274	−8.116	<0.001
Aggressive periodontitis	0.316	0.103	3.082	0.003
African origin	0.614	0.218	2.819	0.006
Female sex	0.291	0.090	3.241	0.002
Body mass index	0.042	0.011	3.925	<0.001

Analysis of variance: p<0.001

Table 7 Backward stepwise multiple regression analysis: lipopolysaccharide-binding protein (LBP) in relation to individual and periodontal parameters

Dependent variable: log-transformed LBP (μg/ml) (n=90)				
R^2=0.165; $R^2_{adjusted}$=0.145; standard error of estimate=0.505				
	b	SE (b)	T	p value
Constant	3.230	0.103	31.456	<0.001
Aggressive periodontitis	0.353	0.117	3.005	0.003
High school	−0.228	0.113	−2.016	0.047

Analysis of variance: p<0.001

in women than in men have been reported before in many [22, 34–36] but not all studies [21].

To our best knowledge, this is the first time that the influence of different types of periodontitis on serum elastase was investigated. After adjustment for age, level of education, pack years, BMI, and African origin serum elastase, CRP [21, 30], LBP, and IL-6 were statistically significantly higher in AgP than in controls. Furthermore, amounts of CRP levels 0.1–0.3 and >0.3 mg/dl were higher in AgP than in ChP. The analysis failed to reveal differences in serum parameter levels between controls and ChP (Tab. 3). However, amount of CRP levels 0.1–0.3 was higher in ChP than in C. Stepwise multiple regression analyses revealed that both serum elastase and CRP are explained by AgP. However, whereas CRP was further influenced by African origin, female sex, and BMI, elastase is negatively correlated to level of education. Increased CRP levels in individuals of African origin (African Americans) in comparison to other ethnic groups (Mexican American, Native Americans, and Caucasians) have been reported before [37, 38]. IL-6 is inducing CRP production in the liver. Both serum parameters are correlated with AgP and BMI. However, whereas CRP is further correlated with African origin and female sex, IL-6 is correlated also with ChP. It seems that the production of CRP despite AgP and BMI depends on different factors than IL-6. Interestingly AgP is still an independent risk factor for elevated serum elastase, CRP, and IL-6 levels after adjustment for sex and age.

Table 8 Backward stepwise multiple regression analysis: interleukin 6 (IL-6) in relation to individual and periodontal parameters

Dependent variable: log-transformed IL-6 (pg/ml) (n=90)				
R^2=0.404; $R^2_{adjusted}$=0.384; standard error of estimate=0.640				
	b	SE (b)	T	p value
Constant	−1.627	0.392	−4.154	<0.001
Aggressive periodontitis	0.723	0.185	3.897	<0.001
Chronic periodontitis	0.862	0.176	4.907	<0.001
Body mass index	0.050	0.017	2.929	0.004

Analysis of variance: p<0.001

The sample size for this study has been calculated with data for serum CRP levels. Respective data for elastase were not available at the time the study was designed. The statistically significant effect of African ethnicity in the multiple regression models is based on the actually small number of four patients with African origin. However, the effect of these four patients is strong enough to be kept in the models.

IL-8 is a key neutrophil chemoattractant. Serum elastase originates from neutrophils. Thus, elevated serum elastase levels may be due to increased serum IL-8 levels. However, whereas elastase is elevated in AgP compared to healthy controls and ChP, the study failed to find differences regarding IL-8.

Due to the fact that it is difficult to find older individuals that are completely periodontally healthy and that ChP as well as AgP were defined by age, the three groups were not well balanced according to age. However, age was not found to influence any of the assessed systemic inflammatory parameters. This is in accordance with other studies that could not demonstrate an association between age and CRP [22]. Interestingly, the BMI of ChP and AgP was similar, though AgP patients were on average 21 years younger than ChP patients. We would have expected a higher BMI in ChP than in AgP because BMI typically increases with age [39]. With a mean BMI of 25.5 kg/m^2 and mean age of 52.8 years, ChP patients are within the regular range of their age group. However, with a mean age of 31.1 years and mean BMI of 26.6 kg/m^2, many AgP patients are in the "higher than normal BMI" group [40]. When considering age, AgP patients tend to be more overweight than ChP patients. Serum CRP levels are strongly correlated to BMI [22, 38, 41]. However, even after adjustment for BMI and ethnicity, AgP is still a significant risk factor for increased serum CRP levels compared to controls and ChP.

The difference between controls and AgP actually may be explained by mean PPD, which is statistically significantly elevated in AgP compared to controls (Table 2). The fact that serum inflammatory parameters (elastase, CRP, and IL-6) are increased in AgP compared to C but not in ChP compared to C cannot be explained by PPD though. In an attempt to describe the whole wound created by periodontal pockets, we added up PPD of all sites per individual. Regarding this parameter, the study failed to find statistically significant differences between ChP and AgP. This indicates that, on average, ChP and AgP patients in this study were similar with regard to size of the "periodontal wound." However, AgP patients responded

with more severe systemic inflammation to the same size of "wound."

Considering elevated serum CRP levels (i.e., moderate, 0.1 to 0.3 mg/dl; high, CRP >0.3 mg/dl [7]) as a risk factor for CHD and taking into account that AgP, at least in most cases, has an earlier onset than ChP (i.e., longer exposure to chronic infection), AgP patients may face a higher risk to suffer from CHD than periodontally healthy people and ChP patients. The detection of gene regions encoding predisposition to CHD and AgP provides additional evidence for such an association [42].

What impact may increased serum elastase levels have on general health? There currently exists some evidence of a weak association between periodontitis and chronic obstructive pulmonary diseases [43]. The development of pulmonary emphysema is explained by the concept of proteases and antiproteases. Under physiological conditions enzymes, e.g., released by neutrophils or periodontal pathogens that cleave elastin and collagen are neutralized by antielastase (e.g., α_1-proteinase inhibitor), which is circulating in the blood in abundance [44]. A chronically elevated serum elastase level may consume a significant amount of antielastase and thereby shift the equilibrium between proteases and antielastases towards destruction.

Within the limitations of the present study the following conclusions may be drawn:

1. Serum elastase and CRP levels in patients with aggressive periodontitis are elevated compared to periodontally healthy controls and patients with chronic periodontitis. Thus, AgP patients have an increased risk for CHD.
2. Serum LBP levels are elevated in AgP compared to healthy controls.

Acknowledgments We wish to thank Dr. Almut von Schwedler, MBBS (Tas), Matcham, NSW, Australia for her valuable and competent support in the preparation of this manuscript.

Conflict of interest and sources of funding statement This study was funded in part by the authors and their institutions as well as in part by grants of the German Society of Periodontology (DGP), the German Society of Dental, Oral, and Maxillofacial Medicine (DGZMK), and the New Working Group for Periodontology (NAgP). The authors report no conflict of interest.

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