Pentraxin-3 detected in human saliva shows limited correlation with biomarkers associated with systemic inflammation

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Pentraxin-3 (PTX3) is a conserved protein of the innate immune system which has been less studied than the pentraxin C-reactive protein (CRP), but it is of relevance in, for example, vascular pathology and pregnancy morbidities. Since the interest in salivary biomarkers in general is increasing, we asked whether PTX3 could be detected in saliva and if any substantial diurnal variation occurs. In addition, we evaluated association with biomarkers of systemic inflammation (interleukin (IL)-1β, IL-6, and IL-8 and CRP), body mass index (BMI), smoking, and age. PTX3 in morning and evening saliva from 106 middle-aged participants of the general population was investigated by ELISA and total protein levels by spectrophotometry. PTX3 was detectable in saliva, and concentrations varied over the day with higher morning concentrations, but the PTX3 relative protein levels (percentage of total protein) were significantly higher in the evening. Sex and age did not impact salivary PTX3, but smoking was associated with lower PTX3 levels. BMI correlated positively with PTX3 in evening saliva. There was no general association with biomarkers of systemic inflammation, except for IL-6. Salivary PTX3 likely reflects the local inflammatory milieu, and adjustments for sampling time, smoking habits, and BMI are needed to adequately interpret PTX3 in saliva.

Key words: Saliva; pentraxin-3; inflammation; biomarker; interleukins.

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The short and long pentraxins constitute an evolutionary conserved superfamily of structurally similar proteins characterized by a pentraxin protein domain [1]. C-reactive protein (CRP) and serum amyloid P component (SAP) belong to the short group, and pentraxin-3 (PTX3) is a long pentraxin [2]. The pentraxins function as pattern recognition molecules sensing foreign antigens and altered self-antigens and tag these for activation of the innate immune system. This property is characteristic of the conserved innate recognition molecules that preceded the development of the immunoglobulins. Although CRP was discovered in the beginning of the last century, our knowledge of PTX3 is still limited [3]. In 1992, Breviario et al. discovered a new gene related to the previously known pentraxins: CRP and SAP [4]. Although PTX3 is structurally and functionally related to CRP, its production differs both regarding its non-hepatic cell origin as well as in its inducing stimuli.

PTX3 is an acute-phase protein and circulating levels can increase rapidly with the peak concentration typically seen 6–8 h after onset of inflammation [3,5]. It is produced by leukocytes, endothelial cells, and fibroblasts during infection and other inflammatory stimulation (e.g., lipopolysaccharide, interleukin [IL-1] β, and tumor necrosis factor [TNF]). However, in contrast to CRP, IL-6 does not promote PTX3 production [6]. PTX3 can also be stored in granules of neutrophils and released during, for example, infections [1,7]. Structurally, PTX3 is a multimeric glycoprotein whose eight
identical protomer subunits comprise 381 amino acids assembling to an octamer with a total molecular weight of approximately 340 kDa [5]. Baseline circulating levels of PTX3 are normally low (<2 ng/mL) [5]. Similarly, to CRP, one of the physiologic functions of PTX3 is to activate the classical complement pathway [8]. Surface-immobilized PTX3 is recognized by C1q and activates the C1 complex at interphases, later leading to activation of the classical activation pathway. On the other hand, fluid-phase PTX3 may consume and inhibit C1q activity. Consequently, PTX3 seems to exert dual roles in the complement system, depending on its presentation [9].

PTX3 has attracted interest in relation to vascular pathology and pregnancy complications [10–15]. Elevated levels of PTX3 have been suggested to predict cardiovascular events, independently of other risk factors, such as increased CRP. In humans, immunohistochemical staining of advanced atherosclerotic lesions revealed a strong tissue expression of PTX3 as well as within the atherosclerotic plaque, combined with plasma level increase rapidly after myocardial infarction, indicating that PTX3 might be a marker of early acute myocardial damage [1].

Mucosal immunity in general, as well as the potential clinical use of salivary biomarkers, is gaining increased attention [16]. Saliva contains a unique mixture of proteins, nucleic acids, electrolytes, and hormones of both systemic and local origin [17]. We have recently investigated CRP in saliva and reported a distinct diurnal pattern of its concentration [18]. In addition, salivary CRP did not straightforwardly reflect circulating CRP levels. To our best knowledge, only a limited number of studies—all with odontological focus—have previously described PTX3 in saliva or in gingival crevicular fluid (GCF) [19–23].

Herein, we primarily aimed to detect PTX3 in saliva and investigate its relation to total protein levels, and if any substantial diurnal variation occurs. Secondly, we sought to evaluate the association between salivary PTX3 and circulating biomarkers of systemic inflammation in collected samples from middle-aged individuals of the general population. Finally, we intended to examine associations between salivary PTX3 and body mass index (BMI), tobacco smoking, and age.

MATERIALS AND METHODS

Study participants

In this cross-sectional study, samples were obtained from 106 participants (60 men, 46 women; median age of 57 years, range 45–69 years) randomly selected as previously described from the Life conditions, Stress and Health Study (LSH) cohort at Linköping University, purposed at investigating the pathways that link psychosocial factors to cardiovascular diseases [18,24]. Exclusion criteria were self-reported severe disease that hindered the possibility to participate, for example, terminal cancer, severe dementia, and psychiatric disorders. Participants with symptoms of infection were instructed to return for sampling after recovery [24].

Collection of saliva

Saliva was sampled at home at 3 time-points over three consecutive days (d1, d2, d3) using Salivette® cotton swabs (Sarstedt AG & Co., Nümbrecht, Germany) as previously described [18]. All participants reported exact time-points in a logbook and were instructed to take samples immediately after awakening (t1), 30 min after awakening (t2), and just before going to bed (t3) following their normal sleeping habits. For the present analyses, only awakening samples (t1) and evening samples (t3), from day 1 (d1) and day 3 (d3), were used. Reported time for the morning sample (t1) was (mean and median) 6:30 AM for both d1 and d3 (Standard Deviation: 57 min). More than 75% of the study participants reported sampling times between 5:30 to 7:30 AM. Around 85% stated that there was <1 h between the two different morning time-points. Regarding evening sampling, most participants went to bed at about the same time every night. Reported time for (t3) sampling was (mean and median) 10:15 PM (Standard Deviation: 65 min). Similarly to the morning sampling, the difference between the two evening time-points was <1 h in ≥85% of the participants. In addition, subjects were told to avoid physical exercise, food intake, and smoking 1 h prior to sampling. After collection, the samples were stored at 4°C until centrifugation. Thereafter, samples were frozen at −70°C until analysis [25]. To standardize for any potential differences in viscosity of the saliva, the samples were centrifuged at 1500 g for 15 min after thawing, and aliquots were taken from the supernatants for upcoming analyses.

Serum CRP

All blood samples were obtained at primary healthcare centers between 6:30 and 9:00 AM (typically between 7:30 and 8:00 AM) in a fasting state of the first day of the week following the saliva-sampling period. Aliquots of sera were stored at −70°C until analysis [24,25]. CRP levels were detected in serum utilizing a highly sensitive latex-enhanced turbidimetric immunoassay (Roche Diagnostics GmbH, Vienna, Austria) with a lower detection limit of 0.03 mg/L, and the coefficient of variance (CV) was 1.7% and the detection rate of 100% [26].

Interleukins in plasma

Levels of IL-1β, IL-6, and IL-8 were measured in EDTA-plasma with ultrasensitive bead kit technology (Invitrogen Co, Carlsbad, CA, USA) on a Luminex® 100TM system (Austin, TX, USA). The lower detection limits were 0.38 pg/mL for IL-1β, 1.68 pg/mL for IL-6, and 0.64 pg/mL for IL-8. The proportions of samples with levels above

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this limit were 50% for IL-1β, 40% for IL-6, and 97% for IL-8.

**BMI and tobacco smoking**

The participants’ BMI was calculated based on weight in kilograms divided by the square of their height in meters (median BMI 26.6, range 19.7–39.5). The separation between ongoing tobacco smokers (17%), prior smokers (35%), and never smokers (48%) was based on self-reported data from the questionnaires as previously described [18]. In some analyses, ongoing smokers were compared with non-smokers (prior and never smokers). Participants who were ongoing smokers also reported dosage (number of cigarettes per day).

**PTX3 in saliva**

PTX3 in saliva was measured in morning (t1) and evening (t3) samples of the third sampling day (d3) using an in-house sandwich ELISA protocol modified from Boij et al. [10]. To avoid potential confounding effects of anti-bovine serum albumin reactivity, human serum albumin (HSA) was used to block non-specific adsorption [27,28]. DuoSet ELISA reagents for PTX3 (capture antibody/842022, detection antibody/842023, and standard curve of recombinant human PTX3/842024) were from R&D Systems (Minneapolis, MN, USA). All incubations were performed at room temperature (RT), using a plate shaker, Corning® Costar 3690 96-well half area polystyrene high-bind microplates (Life technologies AB, Taby, Sweden) were coated with 50 µL of 1 µg/mL mouse anti-human PTX3 capture antibody diluted in carbonate buffer, pH 9.7. The plates were incubated at RT, initially 60 min on a plate shaker and then overnight. The wells were washed with PBS-Tween using a HydroFlex® washer, (Tecan Austria GmbH, Grodőg, Austria) and blocked for 60 min with 150 µL of 20% HSA (Albunorm® 200 g/L; Octapharma, Stockholm, Sweden) diluted 1:20 in PBS. Standard curve with recombinant human PTX3 was diluted in high-performance ELISA buffer (HPE-buffer; Sanquin, Amsterdam, the Netherlands) to gain a maximum concentration 500 pg/mL and an additional 7 times to get a minimum of 3.9 pg/mL. HPE-buffer were used as blank samples. Saliva samples were diluted 1:50 in HPE-buffer. After washing, 50 µL of diluted standard, samples, and controls were added in duplicates to the plate and incubated for 2 h. A biotinylated goat anti-human PTX3 antibody diluted in HPE-buffer was used as detection antibody. After washing, 50 µL of the detection antibody was added to each well and then incubated for two hours. After washing, 50 µL of streptavidin-poly-HRP (Sanquin), diluted 1:10 000 in HPE-buffer, was added to the wells and then incubated for 30 min. After washing, 50 µL of 3.3', 5.5'-tetramethylbenzidine (TMB; Sigma-Aldrich Sweden AB, Stockholm, Sweden) was added and incubated in darkness for 30 min. 1.8 M H2SO4 was used as stop solution; 25 µL was added to each well. The plate was read using Sunrise® microplate reader (Tecan) at 450 nm, with the reference set at 570 nm. Raw data were processed using Magellan software version 7.1 (Tecan). A CV of maximum 20% was allowed for the duplicate data of each sample.

To avoid confounding contributions from blood in saliva, visibly blood-contaminated samples were excluded from further statistical analyses.

**Total protein**

Total protein content was quantified in morning (t1) and evening (t3) saliva of two sampling days (d1 and d3), giving four values for each participant. Concentrations were obtained by measuring the absorbance at 280 nm with a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) using the NanoDrop® ND-1000 software V.3.8.1. A general reference setting was utilized based on the assumption that a 0.1% (1 mg/mL) protein solution produces an absorbance of 1.0 AU (Absorbance Unit) at 280 nm. Amino acids with aromatic rings are the primary reason for the absorbance peak at 280 nm. All samples were analyzed undiluted in duplicates (CV = 1.2%).

**Statistics**

Analyses were performed using SPSS® v.20 (IBM Co., Armonk, NY, USA) and figures made in GraphPad Prism 5.03 (GraphPad Software, La Jolla, CA, USA). p-values <0.05 were considered statistically significant. Plasma interleukin and salivary PTX3 levels below the lower detection limit were given half the limit’s value. To investigate the influence of sex and age on PTX3, Mann–Whitney U ranking analysis was employed. Regarding age, participants <60 years of age (N = 64) were compared to those ≥60 years (N = 42). Correlations between salivary morning/evening PTX3, total protein levels, serum CRP, BMI, dosage of smoking, and interleukins were investigated by Spearman (r.s). Diurnal variations of PTX3, total protein, and PTX3-percentages of total protein, respectively, were investigated by Wilcoxon rank sum tests. To evaluate the performance of salivary PTX3 to identify (biomarker-defined) “systemic inflammation,” the 90th percentile of serum CRP values among the 106 participants was used to define a cutoff. Sensitivity (proportion of samples correctly identified with systemic inflammation), specificity (proportion of samples correctly identified without systemic inflammation), and accuracy (proportion of correctly classified samples) based on the 90th percentile of serum CRP and salivary PTX3 were calculated, including 95% confidence intervals (CI) using the Wilson score method.

**RESULTS**

**General characteristics of salivary PTX3**

Detectable salivary PTX3 levels ranged between 1.57 and 16.1 ng/mL. Median (interquartile range (IQR) = 25th–75th percentile) for morning samples was 2.45 (0.78–5.69) ng/mL and 0.78 (0.78–2.82) for evening samples (Table 1). The highest detected salivary PTX3 levels were unrelated to visible blood contamination. No significant difference in mean PTX3 levels was found between the age groups (Fig. 1A), and sex had no obvious influence on
PTX levels, either in morning or evening saliva or mean levels (Fig. 1B).

Salivary PTX3 versus total protein levels

Total protein levels ranged from 2.58 to 32.8 mg/mL, with a median (IQR) of 8.10 (5.43–12.2) in morning samples, and from 1.46 to 7.38 mg/mL in evening samples with median (IQR) 3.09 (2.53–4.12). A significant substantial positive correlation was found between morning salivary PTX3 and morning total salivary protein (rs = 0.620, p < 0.01, N = 96; Table 2). For evening samples, the correlation to total evening protein levels was less pronounced (rs = 0.247, p < 0.05, N = 101; Table 2). The correlation between salivary morning and evening PTX3 levels was modest (rs = 0.260, p < 0.05; Fig. 2).

Diurnal variation of salivary PTX3 and relation to total protein

The PTX3 concentration in saliva varied over the day with higher absolute values seen in morning than in evening samples (p < 0.0001, N = 91; Fig. 3A,C). However, the PTX3 percentage of the salivary total protein content was higher in the evening than in the morning (p < 0.001, N = 91; Fig. 3B,D).

Salivary PTX3 versus circulating CRP and interleukins

No significant correlation was found between salivary PTX3 and serum CRP, either in morning or in evening samples (Table 2). Four outliers with serum CRP levels above 10 mg/L were identified. The outliers showed varying levels of PTX3 in saliva, ranging from 7.8 to 14.3 ng/mL in morning samples and 1.59–2.38 ng/mL in evening samples.

Detectable IL-1β levels for the subjects ranged from 0.38 to 16.2 pg/mL and the median (IQR) was 0.697 (0.514–1.13). The correlation between plasma IL-1β and morning salivary PTX3 did not meet statistical significance (rs = 0.192), neither did evening salivary PTX3 (rs = 0.006, N = 98; Table 2). Detectable IL-6 levels ranged from 1.77 to 22.9 pg/mL (2.56–5.29). No significant correlations were shown for morning (rs = −0.022, N = 92) or evening samples (rs = −0.171, N = 98; Table 2). However, when only IL-6 levels above the detection limit were considered, a significant correlation was found for morning samples (rs = 0.424, p < 0.05, N = 32). Comparison of PTX3 in evening saliva did not reach statistical significance with IL-6 above detection limit (rs = −0.249, N = 36; Table 2). Detectable IL-8 levels used for analysis ranged from 1.34 to 43.0 pg/mL (4.74–11.6). There was no significant correlation between IL-8 and

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**Table 1.** Descriptive statistics for salivary PTX3 levels in individuals of the general population

|                          | Salivary PTX3 morning (ng/mL) | Salivary PTX3 evening (ng/mL) | Sample pairs morning–evening |
|--------------------------|-------------------------------|-------------------------------|------------------------------|
| Range                    | <1.57–16.1                    | <1.59–10.5                    |                              |
| Mean (SD)                | 3.87 (3.62)                   | 2.16 (2.09)                   |                              |
| Median (IQR)             | 2.45 (0.78–5.69)              | 0.781 (0.78–2.82)             |                              |
| N                        | 96                            | 101                           | 91                           |

IQR, interquartile range (25th–75th percentile); SD, standard deviation.

Blood-contaminated samples are excluded. Levels below the cutoff (1.56 ng/mL) limit were given the value 0.78 ng/mL.

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PTX3 levels, either in morning or evening samples (Table 2).

Association between salivary PTX3 and BMI and smoking habits

The BMI ranged from 18.7 to 39.5, with a median (IQR) of 26.0 (23.3–29.0). No significant correlation was found between morning salivary PTX3 levels and BMI ($r_s = 0.048$, N = 93; Table 2). For evening samples, a significant correlation was observed ($r_s = 0.230$, $p < 0.05$, N = 98).

Regular smoking was significantly associated with lower PTX3 levels in evening saliva, but did not meet statistical significance for morning samples (Fig. 4A). In morning saliva, there was a seemingly but unsignificant gradual decrease in PTX3 levels in never smokers compared to prior and ongoing smokers (Fig. 4B). Likewise, among regular smokers, the reported dosage (number of cigarettes per day) did not correlate significantly with PTX3 levels in either morning or evening saliva.

Specificity and sensitivity of salivary PTX3 to identify elevated serum CRP

By using the 90th percentile of serum CRP values among the 106 participants, a cutoff of 5.0 mg/L was achieved and used to define “systemic inflammation” based on laboratory findings. The CRP data were compared with the 90th percentile of salivary PTX3. As demonstrated in Table 3, the specificity of both morning and evening saliva to detect individuals with biomarker-defined systemic inflammation was decent. However, the sensitivity was poor, particularly regarding evening saliva.

DISCUSSION

This study was undertaken to detect PTX3 in saliva and evaluate any diurnal variation as well as relations to background variables and other biomarkers of inflammation (plasma IL-1β, IL-6, and IL-8 and serum CRP). Detection of PTX3 in human saliva has previously been described, but none of these reports took the amount of salivary total protein levels into account [20–22]. The samples analyzed herein were obtained from the LSH study, originating from a defined group of middle-aged individuals of the general Swedish population. Hence, inflammatory conditions and other diseases could occur [24]. Only self-reported severe illness was an exclusion criterion. The blood-contaminated saliva samples were excluded. In a post hoc analysis, however, it was concluded that blood-contaminated

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**Table 2.** Spearman ($r_s$) correlations for morning and evening salivary PTX3 with other biomarkers and BMI

| Salivary PTX3; morning (Spearman, $r_s$) | Salivary PTX3; evening (Spearman, $r_s$) |
|-----------------------------------------|-----------------------------------------|
| Salivary total protein; morning 0.620** (N = 96) | N/A |
| Salivary total protein; evening N/A | 0.247* (N = 101) |
| Serum CRP −0.023 (N = 94) | −0.059 (N = 99) |
| Serum CRP (≤3 mg/L) 0.076 (N = 76) | 0.057 (N = 80) |
| Serum CRP (>3 mg/L) −0.095 (N = 18) | 0.226 (N = 19) |
| Plasma IL-1β 0.192 (N = 92) | 0.006 (N = 98) |
| Plasma IL-1β* −0.006 (N = 43) | −0.254 (N = 43) |
| Plasma IL-6 −0.022 (N = 92) | −0.171 (N = 98) |
| Plasma IL-6* 0.424* (N = 32) | −0.249 (N = 36) |
| Plasma IL-8 −0.082 (N = 92) | −0.015 (N = 98) |
| Plasma IL-8* −0.071 (N = 88) | −0.052 (N = 93) |
| BMI 0.048 (N = 93) | 0.230* (N = 98) |

BMI, body mass index; CRP, C-reactive protein; IL, interleukin; N/A, not applicable; PTX3, pentraxin-3.

*Above detection limit.

**$p < 0.01.$

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**Fig. 2.** Correlation demonstrated between absolute PTX3 levels in morning and evening saliva.
saliva samples did not substantially alter the results (data not shown).

The correlation achieved when comparing PTX3 in morning and evening saliva was weak but statistically significant. However, a notable observation was the distinct diurnal variation of PTX3 both in terms of absolute measures and in percentage of total protein concentrations. Although the detected PTX3 levels were higher in morning saliva, its proportion of total protein was significantly higher in evening saliva. This finding is new and corresponds only partly with our recent survey of salivary CRP, which was also higher in morning saliva but CRP levels were equal in morning and evening saliva expressed as percentage of total protein levels [18]. Nevertheless, data from studies of other salivary proteins (e.g., α-amylase) indicate that diurnal variation may occur in the same way as for pentraxins [18,29].

Sex did not seem to influence salivary PTX3 levels; in our study, no significant difference was found, either for morning, evening, or the mean of these values. This is in line with previous observations for plasma PTX3 and salivary CRP [12,18,30]. Although it has been concluded that increasing age is associated with higher serum PTX3 levels, age did not appear to influence the salivary levels of PTX3 herein [12]. However, to decide the impact of age, a group of younger saliva donors would have been required. Data on oral health status and salivation rate would have been helpful as well [16].

When establishing a standardized protocol of saliva sampling, the collection method is essential and should be considered when comparing different studies. Mechanical stimulation achieves higher flow rates than the “passive drooling” and can be more comfortable for the donor [31]. The use of Salivette® in the collection of saliva is not an entirely unstimulating procedure, since the instructions allowed the participant to chew on it if needed. Topkas et al. has reported a significant increase in salivary flow using Salivette® cotton swabs compared to their “passive drooling” method. However, total protein concentrations did not seem to change much between these two sampling procedures [32]. The data achieved in the present study rather underline the need to standardize the time point of sampling.

Our investigation did not include measurement of serum PTX3, which is a limitation. The only

Fig. 3. PTX3 levels in morning and evening saliva. Displayed data are absolute PTX3 levels (A) and PTX3 levels relative to the total protein concentrations (B). Related morning and evening absolute PTX3 concentrations (C) and percentages (D) are shown for each participant. Significant differences are highlighted by **p < 0.01.
study that we found which compared PTX3 levels in serum with saliva did not identify any significant correlation [20]. However, the study population in the Turkish study included individuals with different degrees of periodontitis why the conclusions may not necessarily be generalizable to other groups. Another study of periodontal health and disease demonstrated an impressively strong correlation between PTX3 in GCF and plasma [23].

Regarding the association between circulating CRP and salivary PTX3 levels, the literature reveals contradictory results and both significant positive correlation and no correlation have been reported. The discrepancies are possibly due to that different conditions were studied [12,33–35]. Herein, serum CRP and salivary PTX3 were not significant regardless of if morning or evening samples were compared. Neither did salivary PTX3 show an impressive sensitivity to identify individuals with biomarker-defined systemic inflammation (elevated serum CRP).

As a main inducer of PTX3, one would expect that IL-1β should be correlated with salivary PTX3. However, we did not find such associations either in morning or evening saliva. We identified one previous study that evaluated the association between salivary PTX3 and serum IL-1β, but the correlation did not meet statistical significance [20]. It should be noted though that IL-1β levels in our study were measured in the circulation while salivary PTX3 constitutes a protein most likely produced locally in the oral cavity [19]. Subsequently, plasma IL-1β levels might be of uncertain importance for the local PTX3 production. Instead, plasma IL-6 (which is not an inducer of PTX3) unexpectedly correlated significantly with PTX3 in morning samples. The latter could potentially be related to poor oral health status, for example, periodontitis.

BMI and smoking are both associated with systemic inflammation [36]. Some studies observed significant associations between salivary CRP and BMI, whereas other did not [18,37,38]. Hitherto, results are inconclusive regarding the association between BMI and circulating PTX3. Inverse correlations between PTX3 levels and BMI, as well as waist circumference, have been reported, whereas another report found increasing PTX3 levels in association with metabolic syndrome parameters (abdominal obesity is one of the components) [33,39]. Karakas et al. suggested that an increasing number of metabolic syndrome components may enhance inflammation and result in rising PTX3 levels [33]. We observed no clear correlation between BMI and morning values, but PTX3 in evening saliva showed a modest direct significant correlation with BMI.

Tobacco smoking appeared to influence salivary PTX3 levels, and the effect was seemingly stronger in evening than in morning samples. The higher

![Fig. 4. Salivary PTX3 levels in relation to self-reported smoking habits shown for morning (M), evening (E), and mean values, respectively (A, B). Significant differences are highlighted by *p < 0.05 or **p < 0.01.](image)

**Table 3.** Performance of salivary morning and evening PTX3 to identify “systemic inflammation,” defined by the 90th percentile of serum CRP values. 95% confidence intervals in parentheses

|                  | Sensitivity | Specificity | Accuracy |
|------------------|-------------|-------------|----------|
| Salivary PTX3 morning (N = 97) | 0.13 (0.13–0.36) | 0.91 (0.86–0.92) | 0.85 (0.80–0.87) |
| Salivary PTX3 evening (N = 103) | 0 | 0.89 (0.84–0.91) | 0.81 (0.76–0.83) |

No confidence interval calculated when sensitivity equals zero.
salivary PTX3 among non-smokers was unexpected but agrees with our recent finding for salivary CRP [18]. In addition, anti-inflammatory properties of nicotine have been documented in obesity and nicotine-mediated modulation of hypothalamic-pituitary-adrenal axis activity was suggested as a potential mechanism [40,41].

Limitations of the study should be mentioned. We acknowledge the lack of data on serum PTX3, oral health status, and salivation rate, which should be considered as limitations. Also, the definition of “systemic inflammation” was based on serum CRP levels only (no physical examination of the participants was performed). Still, all individuals with symptoms of infection or severe disease were excluded [24].

To conclude, while the rationale is strong to investigate salivary PTX3 in relation to circulating biomarkers of systemic inflammation, we could not confirm coherent associations in collected samples from middle-aged individuals of the general population. We observed variation of salivary PTX3 over the day with higher absolute levels in morning saliva, whereas PTX3 levels expressed as percentage of total protein concentrations were significantly higher in evening saliva. We found no clear impact of sex and age, but tobacco smoking was associated with decreasing PTX3 levels, particularly in evening saliva. BMI correlated significantly with PTX3 in evening saliva. The sensitivity of salivary PTX3 to detect individuals with elevated serum CRP was poor. Thus, adjustment for factors such as sampling time, smoking, and BMI is needed to be able to adequately interpret PTX3 in saliva. These circumstances will probably limit the usefulness of salivary PTX3 as a biomarker in clinical routine.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. J. W., F.J., and C.S. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: J.W., M.K., P.G., C.S. Acquisition of data: F.J., M.K., P.G. Analysis and interpretation of data: J.W., F.J., M.K., P.G., C.S.

ETHICAL STATEMENT

Oral and written informed consent was obtained from all participants. The LSH study protocol was approved by the regional ethics review board in Linköping (No. 02–324).

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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.

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