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Review

Towards salivary C-reactive protein as a viable biomarker of systemic inflammation

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ABSTRACT

C-reactive protein (CRP) is a commonly used marker of systemic inflammation, routinely measured in serum blood samples. However salivary samples offer a non-invasive and easily accessible alternative which would improve point of care (POC) testing for inflammation. Two major challenges restrict the use of saliva: the influence of the oral environment on CRP and its local production; and collecting a standardised sample given patient-dependent salivary flow rates. Here we review the reported studies of salivary CRP in humans as a potential marker of systemic inflammation and how the challenges can be overcome. Salivary CRP currently poorly reflects systemic inflammation as it does not consistently and strongly correlate with serum CRP. The mean and one standard deviation reported R^2 values are 0.53 ± 0.23 from 14 studies. An improved understanding of the key challenges and implemented solutions are needed to optimise salivary CRP use. Firstly, control for the effects of local oral inflammation. Screening for oral trauma is one option, however this could drastically limit the number of patients suitable for salivary CRP testing and the number of professionals able to use the POC test. Secondly, the role of a dilution biomarker is considered controlling for salivary flow rate which dilutes serum CRP by $\sim 10^4$; a variable and likely-patient specific factor. The ideal dilution biomarker should have many of the pharmacokinetic, sensitivity and specificity characteristics of CRP. The potential for positive acute phase protein serum amyloid A (SAA) and negative acute phase protein albumin is considered and the characteristics of any correction function discussed. Currently, however, there are no available strategies to make salivary CRP a reliable quantitative measure of serum CRP and hence POC systemic inflammation testing.

1. Introduction

Serum is the preferred biofluid for biomarker measurements diagnosing and managing a range of pathologies including inflammation [1,2]. However, recent advances in proteomics have highlighted the diagnostic potential of other biofluids, such as the use of cerebral spinal fluid in diagnosing Parkinson's disease and urine in investigating stable angina [3–5]. Saliva has received considerable attention as it has many advantages over blood plasma. For example, it can be collected *via* a simple, non-invasive method which does not require specialist training or equipment and places the collector at reduced infection risk compared to blood plasma collection. This makes it more suitable for repeated sampling, collection from difficult patients and in non-clinical settings [6–10]. Overall, this could make saliva an ideal means of systemic inflammation testing at POC, which could greatly improve the management of many pathologies, particularly inflammation and scenarios where repeated sampling would be of value such as in post-operative recovery to allow early detection of a complication and early intervention.

The potential benefits of saliva have resulted in increased research leading to some established clinical uses, for example, in assessing cortisol and immunoglobin A levels and screening for human immunodeficiency virus *via* a POC device [8,11]. Furthermore, many potential uses of saliva, such as in diagnosing breast cancer, autism, cardio fitness and body mass index are under investigation [12,13]. However, before saliva-based diagnostics can be routinely used, multiple obstacles need to be overcome. The three major challenges are: the influence of the oral environment on CRP, collecting a standardised sample and extracting biomarkers reproducibly from the complex saliva matrix [7,11,14–16]. Overcoming these challenges may enable saliva to be fully utilised, dramatically improving the ability to diagnose and manage pathologies.

1.1. Saliva

Saliva is a complex biofluid located within the oral cavity which

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may reflect systemic health [17]. It is comprised of many components including salivary exocrine secretions, gingival crevicular fluid (GCF) and microbes [10,18,19]. GCF is a serum exudate that carries all of the major components of the blood including Complement proteins, immunoglobins, cells such neutrophils and plasma cells and the acute phase proteins such as CRP and Serum Amyloid A [20,21] Salivary exocrine secretions make up the majority of oral fluid and are primarily secreted by the parotid, submental and submandibular salivary glands [18]. Salivary secretions include water, mucus and analytes such as immunoglobins and cortisol, which serve many functions for example in aiding digestion and taste [6,18,22,23]. Whilst GCF may reflect the blood plasma composition, the additional secretions from the three salivary glands dominate the plasma composition in two ways: 1) simple dilution; and 2) the saliva matrix may trap proteins which need to be recovered accurately prior to analysis. Consequently, the composition of the saliva may not reflect that of the plasma depending on the degree of salivation or simulated salivation during collection and the location in the mouth: small sample volume may show region specific concentrations. Characterisation of the salivary proteome has highlighted that saliva is a promising biofluid with potentially multiple clinical uses, by detecting numerous serum biomarkers within saliva such as CRP and transferrin reflecting the blood proteome and the systemic acute phase response, Fig. 1 below [5].

1.2. Inflammation and CRP

CRP is a key marker of inflammation within clinical practice. It is a pentameric 125.5 kD positive acute phase protein (APP) that mediates innate immunity by bonding to foreign pathogens and damaged cells, triggering the Classical Pathway of the Complement cascade. It consists of 5 subunits each with a molecular weight of 25.106 kD [15,24–26]. Intact CRP has a mass above the cut-off limit for renal excretion (43 kDa) and is a circulating biomarker of inflammation [27]. CRP has been observed in the urine, especially during renal failure where the cut-off may be compromised. It is important to not however that all immune-assays formally the assay detection epitope which if present on the monomer, would not imply a degradation of the filtration cut-off. Most manufacturers are not clear about the location of the detection epitope in a detection assay and the reported studies are unclear on the location of the epitope in theirs assays.

Upon production, serum CRP levels rise up to 50,000-fold from baseline, doubling every 8 h and peaking approximately 42 h after the initial stimulus [1,2]. Alternative stimuli result in different CRP responses. For example, viral infections or mild inflammation causes serum levels between 10 and 40 mg/L, whereas severe bacterial infections and major trauma cause serum CRP levels to be greater than 300 mg/L [28]. As serum CRP remains in the circulation and has a predictable response to inflammatory stimuli it is the most commonly used clinical method for assessing inflammation [1,2].

1.3. Hypothesis

Considerable research has been performed into whether salivary CRP has clinical potential. Investigations focus on whether salivary CRP levels correlate with its serum counterpart and the presence of pathologies. However, no review has focussed on the evidence to determine whether salivary CRP could be good marker of systemic inflammation and how salivary limitations may be overcome [6,22,29–39]. This review aims to determine whether salivary CRP is a good biomarker of systemic inflammation. It critically analyses the detected correlations between serum and salivary CRP reported to date and the major challenges restricting the use of salivary CRP of the influence of the oral environment on CRP and collecting a standardised sample. Developing our understanding of these challenges may highlight solutions and guide further research which could make salivary CRP a better marker of systemic inflammation. Solving these challenges is essential if salivary CRP is to become a good marker of systemic inflammation, which could revolutionise the management of inflammation.

2. CRP presence within saliva and correlation with serum CRP

To determine if salivary CRP is a viable biomarker of systemic inflammation, a correlation between salivary and serum CRP levels needs to be established. The correlation would ideally be linear but could be of any order although non-linear correlations would be difficult to identify accurately. Arguably a pure GCF sample would show the highest correlation with serum levels with no reported mRNA for CRP production locally; the correlation degrading with the presence of other secretions and local inflammation recruiting CRP or potentially non-

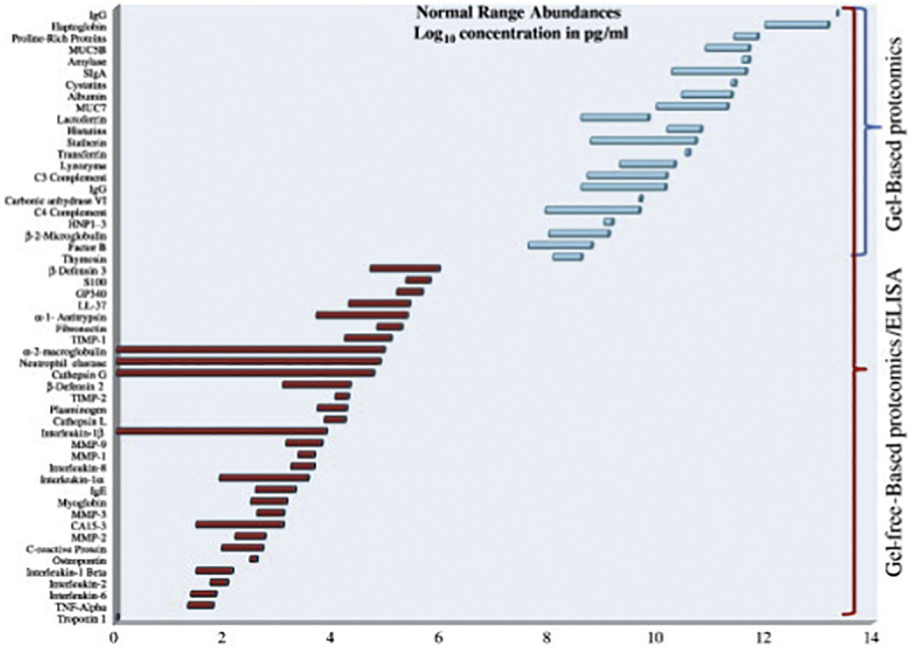

Fig. 1. Relative abundance of 50 different proteins located within the saliva of healthy subjects. Relative prevalence is represented on a log scale, which spans 14 orders of magnitude is represented on the abscissa. Gel-based and non-gel based proteomic technologies were utilised to calculate the relative abundance of the proteins [5]. (Image reproduced with permission from the rights owner).

Table 1
Correlation coefficients from the selected studies identifying the cohort, size, the mean saliva and serum CRP concentrations and the saliva/serum ratio – the serum dilution factor.

Correlation coefficient	Cohort	Reference
$R^2 = 0.84$	$N = 28$ Cardiac Patients $N = 55$ healthy patients; Paired serum-saliva CRP samples Mean Serum = 33 mg/L Mean Saliva = 285 pg/mL (healthy), 1680 (cardiac) pg/mL Serum/Saliva ratio = 1.1×10^5	[22]
$R^2 = 0.289$	$N = 28$, Acute MI patients, $N = 28$ Healthy Controls	[29]
$R^2 = 0.249$	Mean Serum CRP healthy individuals (3.84 ± 0.60) mg/L Mean Saliva 0.8 ng/mL (unstimulated) Mean Saliva 0.3 ng/mL (stimulated) Serum/Saliva ratio = 4.81×10^3 (unstimulated) Serum/Saiva ration = $1.2\ 1 \times 10^4$ (stimulated)	
$R^2 = 0.86$	$N = 100$ COPD Patient cohort Mean Serum CRP = 90 µg/mL Mean Saliva = 10.7 ± 4.8 µg/mL Serum/Saliva ratio = 9	[30]
$R^2 = 0.62$	$N = 100$ Control cohort Mean Serum mean = 0.45 ± 0.72 µg/L Mean Saliva CRP 0.74 µg/L Serum/Saliva ratio = 0.6	
$R^2 = 0.01$	$N = 55$ Healthy Adults Median Serum (plasma) CRP = 2.0 mg/L Median Saliva CRP 1.2 µg/L Serum/Saliva ratio = 1.7×10^3	[31]
$R^2 = 0.424$ ($R^2 = 0.599$ for high CRP)	$N = 18$ Healthy Adults Mean Serum CRP = 2.24×10^4 ng/L Mean Saliva CRP = 0.08 ng/mL Serum/Saliva ratio = 2.8×10^5	[32]
$R^2 = 0.20–0.70$	$N = 73–122$ healthy adults and at risk of cardiovascular disease longitudinal study Mean plasma CRP 3.5–4.3 mg/L (over the time course) Mean saliva CRP 0.0026–0.0063 mg/L (over the time course) Serum/Saliva ratio = 1346–682	[33]
$R^2 = 0.424–0.599$	$N = 35$, 17 depressed adolescents and 18 healthy controls Mean plasma CRP 2.24×10^4 ng/mL Mean salivary CRP 0.08 ng/mL Serum/Saliva ration = 2.8×10^6	[34]
$R^2 = 0.66$	$N = 781$ Post-menopausal women Mean Serum = 2908 ng/mL Mean Saliva = 0.992 ng/mL Saliva/serum = 2.9×10^3	[35]
$R^2 = 0.73$	$N = 259$, arterial stiffness and subclinical atherosclerosis Mean Serum 1.05 mg/L Mean Saliva 0.21 ng/mL Serum/Saliva ratio = 5×10^3	[36]
$R^2 = 0.62$	$N = 40$ neonates Mean Serum = 106 µg/mL Mean Saliva = 3.1 ng/mL Serum/saliva = 3.4×10^4	[38]
$R = 0.41$	$n = 61$ healthy adults Mean serum = 2.02 mg/L Mean saliva = 2994 pg/mL Saliva/Serum 1633 Note R^2 value is re-derived from the Fig. 1 in the reference which report a Pearson correlation coefficient $r = 0.72$.	[39]
$R^2 = 0.66–0.8$	$N = 21$ alcohol septal ablation and $n = 97$ healthy adult controls Longitudinal study Mean Serum = 50,000–100,000 ng/mL (estimated) Mean Saliva = 5–10 ng/mL (estimated) Saliva/Serum = 10^4	[41]
Mean $R^2 = 0.53 \pm 0.23$	Mean of values or mid-range values with one standard deviation	

hepatic synthesis [21,40]. However, collection of GCF samples is considerably more difficult, requiring specialist training and equipment, limiting the suitability of this investigation tool.

Multiple studies have assessed the correlation between salivary and serum CRP levels; fourteen studies are shown in Table 1 where the correlation has been reported explicitly or could be derived from the data. The studies varied considerably in their methodology, specifically whether they analysed the salivary-serum relationship in healthy participants, or subjects with varying sources and levels of inflammation. Most detected a positive correlation, however three studies found no correlation. Furthermore, the correlation coefficient strength varied considerably between and within studies [6,22,29–36,38,39,41]. For example, the highest correlation of 0.86 was found between the salivary and serum CRP of hospitalised COPD patients. However, the control group had a lower correlation of 0.62 [30]. Despite inconsistencies, overall the literature indicates a moderate correlation between salivary and serum CRP and a serum dilution factor of order 10^4 [6,22,29–36,38,39,41]. The mean and standard deviation R^2 values are 0.53 ± 0.23; a 95% confidence limit on R^2 is 0.07–0.99 – the correlation is not well established.

Correlation strength also varied depending on the serum CRP level. Four studies found that the salivary-serum CRP correlation was greater when serum levels were elevated [30,34,36,39] probably due to relatively higher percentages changes in dilution. Understanding the causes of the correlation and dilution variances in the studies is important to determine if and how the salivary-serum CRP correlation can be improved, to achieve consistent correlations of 0.7 which is considered by some to be a strong correlation [42]. From here, the coefficient of variation can be calculated. A coefficient of variation below 20% indicates that salivary CRP would be a suitable replacement for serum CRP [43]. To strengthen the salivary-serum CRP correlation and enable salivary CRP to become a better marker of systemic inflammation, four key challenges need to be overcome.

3. Challenge one – the influence of the oral environment on CRP

The local environment perturbs salivary CRP as a reflection of systemic inflammation with mechanisms local inflammation sites and differential CRP clearance half-life [6,22,31,32,44,45]. Consequently, understanding and overcoming these limiting factors might improve the use of salivary CRP as a marker of systemic inflammation.

3.1. Salivary CRP half-life

The half-life of salivary CRP is yet to be determined and must be different to the stable and well categorized half-life of serum CRP of 19 h associated with hepatic clearance [1,2]. The environment within the oral cavity is highly variable and differs significantly from blood plasma. For example, the oral cavity is more alkaline and may contain many contaminants such as bacteria, food products and degraded proteins [22,45]. It is therefore highly likely that salivary CRP is not as stable and does not share the same half-life as serum CRP. Mirzaii-Dizgah et al. produced findings in myocardial infarction patients which

indicated a reduced half-life of salivary CRP as levels began to return to baseline prior to serum levels. However, they did not determine the exact half-life of salivary CRP and only used two collection points over a 24-h period to compare the time taken for salivary and serum CRP levels to return to baseline [29]. Therefore, to confirm this trend and allow comparison with the half-life of serum CRP, salivary and serum CRP levels need to be measured at more frequent time intervals.

3.2. Impact of local inflammation

The presence of inflammatory stimuli within the oral cavity may result in salivary CRP levels which are unrelated to the degree of systemic inflammation present. Localised inflammation could result in recruitment to the oral cavity, increased GCF secretion rates and the risk of bleeding into the saliva [46,47]. In the three studies which did not find a significant salivary-serum CRP correlation, the lack of control for oral health may have caused salivary-serum CRP levels to be unrelated [6,31,32]. However, other studies, that also did not control for oral health, found significant correlations [29,36,38,41]. The difference between these groups of studies is that the three studies which found no correlation used only,or predominantly, healthy volunteers [6,29,31,32,36,38,41]. Two recruited only healthy volunteers and the third had over double the number of control participants compared to subjects with suspected pulmonary tuberculosis [6,31,32]. As there was a significantly greater number of controls, the healthy population would have had a larger bias on the salivary-serum CRP correlation. Therefore, localised inflammation may only influence the salivary-serum CRP relationship in the absence or limited presence of systemic inflammation. If localised inflammation produces inaccurate results, the clinical utility of salivary CRP may be systematically limited due to the production of falsely high results.

Significant levels of systemic inflammation could dominate the local oral cavity production. Redman et al., found that salivary and serum CRP levels remained correlated in rheumatoid arthritis or osteoarthritis sufferers who had periodontitis, a chronic inflammatory disorder of the gums that increases salivary CRP levels [16,48,49]. They concluded that CRP elevations induced by periodontitis were overshadowed by the presence of pathologies such as rheumatoid arthritis or osteoarthritis inducing inflammation [49]. The impact of local inflammation on salivary CRP needs clarification in both healthy participants and subjects with elevated serum CRP to validate the need for control of oral trauma within the literature.

Understanding the influence of oral inflammation is important as it is a common comorbidity. One study, performed in the US between 2009 and 2010, found that 47% of the population suffered from periodontitis, most of which was of moderate severity [50]. Furthermore, hospitalised patients have been found to have worse oral health when compared to outpatients. This could be the result of a higher likelihood of hospitalised patients having other comorbidities and medications which may alter the environment within the oral cavity [51]. These factors are important as this cohort is more likely to warrant salivary CRP testing. Consequently, if the presence of localised inflammation means that salivary CRP testing is unsuitable, this severely limits the clinical utility of salivary CRP as a large cohort of subjects would be unsuitable for this investigation procedure.

However, not all causes of localised inflammation may elevate salivary CRP levels. Gingivitis, inflammation of the gingival tissue which may progress into periodontitis, has been confirmed to have limited effect on salivary CRP levels in two studies [16,52]. However, these studies used small cohort sizes and one found an insignificant elevation which may have become significant with a larger cohort [16,52]. Developing our understanding of how different types of localised inflammation influence the salivary-serum CRP relationship is essential. It may be that subjects with certain types or degrees of localised inflammation are suitable for this investigation, increasing salivary CRPs diagnostic utility. Further studies are required to understand if any localised inflammation disproportionately influences salivary CRP levels by recruitment, local production or bleeding, this would make salivary CRP a poor marker of systemic inflammation.

3.3. Screening for localised trauma and inflammation

One solution to limit the influence of localised trauma on salivary CRP levels is to screen for oral inflammation. A range of different screening methods is currently in use. For example, one study used a brief questionnaire to assess oral health, whereas another performed clinical dental examinations including oral radiographs [33,35]. A dental examination is more representative than a self-reported questionnaire. However, a dental examination is considerably more specialist, time consuming and invasive to perform, which would dramatically reduce the practicality of salivary CRP testing and its attraction as a non-invasive measurement. Self-reporting questionnaires found a stronger salivary-serum CRP correlation, however, there may be confounding factors such as analysis technique [22,35,39]. The development of a standardised, simple, quick and accurate method of screening for localised inflammation is essential for creating an investigative tool that maximises the benefits of salivary CRP and enables greater comparisons between studies.

4. Challenge two – collecting a standardised sample

In order to increase the ability of salivary CRP to monitor systemic inflammation, a consistent collection method that optimises salivary CRP levels is required. Currently, no consistent method is used and clearly reflects differential dilution in the oral cavity, a key challenge [22,33–36,38,41,53,54]. Many other areas require research and standardisation, such as the need to control for the level of stimulation, collection location and technique, however, until further research is performed these can all be controlled by applying consistent control methods [6,19,22,29–39,54–58].

4.1. Control of salivary flow rate

One key factor influencing saliva samples is effectively controlling the variable flow rate of saliva [59]. Salivary flow rate is influenced by many variables including the level of stimulation present, pharmaceuticals and patient comorbidities such as diabetes [60,61]. Furthermore, there is considerable variability within healthy individuals. For example, one study of 25 healthy individuals between the ages of 20 and 35, found unstimulated flow rate to vary by almost 50% of the average flow rate [54].

Despite the development of multiple methods to control for variable flow rate, none of these have yet provided a suitable solution. Controlling *via* total salivary volume has been used to normalise analyte levels in multiple studies. However, this method provides a weaker correlation with CRP levels than other methods [38]. Furthermore, this method may be unsuitable for POC devices as it requires the collection of large quantities of saliva for assessment. Calculating salivary flow rate to normalise samples also suffers this same flaw as it requires large sample volumes collected over a prolonged time period [38]. Moreover, three studies found that salivary CRP does not correlate with salivary flow rate, indicating that this control method is inadequate [31,39,62]. However, it may also highlight that it is not necessary to control for salivary flow rate. All three studies used healthy individuals and controlled for the level of stimulation present, which did not improve the correlation. It is possible that there may not have been enough variation to detect discernible differences in CRP levels for correlation [31,39,62].

Measuring the total protein concentration alongside analyte levels provides a potential dilution control method and would be the comparatively straightforward to implement in a POC device and has been shown to improve the correlation [38]. Total protein levels would also

be vulnerable to the same problems associated with local trauma and variable levels of stimulation whereby the protein composition can vary significantly [63–65]. Therefore, similar protein concentrations could have a very different protein composition, meaning that total protein concentration may not accurately reflect salivary CRP levels.

4.2. Development of a dilution marker

Dilution factors of order 1.6×10^4 have been reported coupled with the 50% variation of reported salivary flow rates, demonstrates the magnitude of the dilution problem [39]. The mean correlation between salivary and serum CRP is $R^2 = 0.53 \pm 0.23$ (mean and standard deviation): small patient variations form day-to-day as well as patient to patient of the large dilution factor could degrade the correlation and its predictive value for serum CRP concentrations. There may be sufficient diagnostic value in identifying CRP-positive saliva but the ideal diagnostic value would be a quantitative measure of salivary CRP to predict serum CRP to invoke all of the diagnostic thresholds and trends that are well established in many disease states.

One solution to overcome the variable salivary flow rate and patient variability in dilution could be simultaneous detection of another analyte or panel of analytes, alongside CRP, within samples that is known to be derived from the serum, referencing the CRP level to the systemic level – a dilution marker. A function of two or more analytes dilution markers could be derived from which the serum concentration could be inferred quantitatively. There are a number of characteristics of a dilution marker or combinations of markers:

1) the biomarker should be derived from the serum reflecting systemic inflammation;
2) the biomarker should ideally remain constant for all levels of serum CRP in all pathophysiological conditions;
3) if the biomarker concentration is not constant should have known fluctuations or definable variations strongly related to those of CRP under all pathophysiological conditions;
4) the biomarker should have a similar rate of production and half-life to salivary CRP to follow faithfully the serum concentration;
5) the biomarker should to enter the oral cavity by the same mechanism as CRP to control for the impact that the collection method and local trauma has upon the results; and
6) any differential local confounding mouth-specific factors need to be detailed.

These are severe constraints on the properties of any one dilution marker but a quantitative dilution marker strategy is more likely to reflect more accurately the serum CRP levels than a correlation coefficient or an average dilution factor correction.

A first set of possible makers may be found in the APP, for example, Serum Amyloid A levels increase up to 1000-fold from baseline in 24–48 h similar to CRP, whereas haptoglobin levels increase 3-fold over a period of 3 days; some properties of other APPs are shown in Table 2 [66,67]. APPs tor combinations therefore may be suitable dilution markers as they change in the presence of the same inflammatory stimuli as CRP and are, principally, synthesised *de novo* in the liver. The two extreme cases are SAA which behaves in a similar way to CRP rising rapidly during the acute phase response and albumin which is a negative acute phase protein, slow to respond and has a moderately low dynamic range.

SAA may be a suitable dilution marker because of its similarities to CRP and relative ease to measure. However, significant uncertainties exist within its serum form [68], it is however relatively easy to measure. SAA is a family of apolipoproteins that transport phospholipids to damaged tissues. Phospholipids then assist in tissue regeneration and recruit inflammatory cells to the sites of inflammation [1,66,69]. SAA responds to the same inflammatory stimuli, such as septic shock, myocardial infarctions and physical trauma in a similar fashion to CRP [66,67,70,71]. Specifically, CRP and SAA share co-stimulation by IL-6, large dynamic range, rapid response in the first 24 h – 48 h, a comparable time taken to reach maximum concentration, return to baseline and serum half-life [1,66,67,71–75]. Variations within these exist, for example SAA takes longer to return to baseline [1,67,72,73]. However, the differences appear to be small.

Table 2
APR response to inflammation (adapted from reference [66], reproduced with permission).

Protein	Acute-phase response	Peak level	Percentage change (%)
CRP	Increase	48–72 h	50,000%
SAA	Increase	48–72 h	100,000%
Haptoglobin	Increase	72 h	300%
$Alpha_1$-Acid Glycoprotein	Increase	72 h	150%
Fibrinogen	Increase	5 days	200%
$Alpha_1$-Antitrypsin	Increase	5 days	200%
Ceruloplasmin	Increase	14 days	130%
Immunoglobulin M	Increase	30 days	130%
Transferrin	Decrease	3–7 days	10–80%
Albumin	Decrease	3–9 days	10–80%
Transthyretin	Decrease	5 h	50%

Whilst it might be hoped that SAA tracks CRP closely, there is reasonable evidence to suggest there is differential response of SAA to CRP depending on the origin of the infection [72,76]. Furthermore, it is unclear if salivary SAA reflects serum levels, verified only in animal models [37,77,78]. The only human study performed on salivary SAA detected no significant elevation in the presence of inflammation [79]. Therefore, clarification over how human salivary SAA responds to systemic inflammation and relates to CRP is required. Finally, before SAA can be used as a dilution marker, there needs to be clarification over how it enters saliva and whether salivary SAA reflects serum levels. SAA is a lipophilic molecule of between 11.4 and 12.5 kDa, therefore it may enter the oral cavity *via* active transport and transudation, which is different from CRP [60,80,81]. Moreover, SAA may be produced locally as mRNA has been detected within human tonsillar tissue [82]. Further confirmation of these entry methods is required as the presence of SAA within the oral cavity could be dependent on different factors to CRP. Consequently, whilst initially promising, SAA may not correlate with CRP or represent serum levels and would be an inadequate dilution marker on its own.

SAA is a rapidly varying protein tracking CRP; the alternative is a protein with a smaller dynamic range or falling during infection such as albumin or transferrin. Albumin is one of the most abundant proteins found within human serum and functions in maintaining osmotic pressure and transporting proteins, such as fatty acids and steroids [4,83,84]. In the presence of some inflammatory stimuli, serum levels decline by between 10% to 80% to allow for an increased production of positive APPs but over a period of 3–9 days [1,66,85]. However, a promising feature of salivary albumin, is that in the presence of systemic inflammation its levels remain unchanged [51,61,86,87]. This may be because salivary levels are much lower and therefore detecting significant differences is more challenging. A non-significant change could make salivary albumin exceptionally useful as the differences in the half-life, time taken to peak and return to baseline between CRP and albumin become unimportant, hence the albumin-CRP ratio would only be dependent on CRP levels [51,66,67]. However, salivary albumin has several flaws restricting its use as a dilution marker. For example, it has a high degree of inter-patient variation in healthy individuals [88,89]. Salivary albumin levels are also elevated in the presence of oral inflammation, although the prevalence of such conditions in the population is unknown and may have an acceptable false-measurement rate [51,61,87].

5. Discussion

Salivary CRP would have significant clinical value as a biomarker of systemic inflammation due to it being derived from a non-invasive biological sample. It could be measured simply at point-of-care and more frequently to provide trend information rather than absolute single-point measures. There are however significant challenges in recovering analytes reproducible from the complex matrix that is saliva and the mechanism by which molecules enter the saliva from the blood. Ideally, a simple correlation would allow the salivary CRP to be corrected to the serum CRP by a simple correlation but the current set of 12 studies, shows an average R^2 of 0.53 ± 0.23 (mean and standard deviation), see Table 1. However, some studies report levels of correlation as high as 0.86 which suggest the search for a reproducible measurement protocol could deliver the required correlation.

5.1. Improving the salivary CRP-serum CRP predictive value

The current observed variation in the correlation between serum and salivary CRP may be explained by (at least) two major factors: changes to the CRP concentration caused by localised inflammation in the mouth and difficulties in collecting a consistent saliva sample. Other factors include working with the saliva matrix separating the proteins reproducibly, the collection protocol, the mode of entry of the serum proteins into the saliva – is this active or passive and is there a cut-off. Most importantly if the non-invasive sampling is to be exploited, a point-of-care test to perform the analysis with a sufficient sensitivity to measure the very low concentrations of CRP, ~2 μg/L.

The impact of local trauma on CRP concentration is a significant challenge. A significant number of studies have evidenced the effect of trauma [6,16,29,31,32,36,38,41,49,52]. This is important as if a successful control could not be found, the utility of salivary CRP testing would be limited to a small subset of the population. The obvious solution is a clinical examination using some form screening tool based on an examination and clinical judgement. The clinical screening tool would however be limited by a visual inspection which will not be sufficiently sensitive or specific to make an accurate, quantitative correction of the salivary CRP level. Improving the sensitivity and specificity of the screening tool would mean an increase in complexity, could require specialist training, increasing cost, restricting collection to only trained personnel and defeating the utility of a low-burden biosample. This is the biggest limitation in the use of salivary CRP as a marker of systemic inflammation and therefore, needs prioritising in salivary CRP research. If it is found to be unsuitable due to the impracticalities of screening and the low percentage of patients who are suitable for the test, then further research to overcome the other challenges would be inappropriate. Fundamentally, a control measurement should be made simultaneously with the CRP to eliminate any inspection and localise the CRP concentration to the systemic blood.

The second major challenge is developing a suitable method of controlling for the dilution of saliva. This may yield the greatest improvements in the salivary-serum CRP relationship as the dilution of saliva is highly variable and the current available methods to control it are ineffective and unsuitable for a POC device [38,54,60,61]. The use of a dilution marker may prove to be the most promising solution but the challenge is the quantitative accuracy of the correction. Ideally, a robust monotonic (single-valued over the diagnostic range of CRP) correction should be made to allow the serum CRP diagnostic thresholds to be mapped to the salivary concentration. The APP themselves perhaps in combination are interesting as detailed for SAA and albumin but these come with problems that need to be understood. Measurement of several APPs could allow the effect of dilution as well as local inflammation to be addressed. A salivary correction function based on an acute phase signature would show the rise in the systemic inflammation with a local inflammation patterns superposed. A dilution biomarker panel could exploit the differential local inflammation effects of say SAA over CRP and provide a discrimination between local and systemic effects. The biomarker panel correction function is likely to be complex and non-linear and would need considerable validation.

The patient variability in the dilution factor and any dilution biomarker is a considerable unknown. An inert protein biomarker such as IgD with no known clinical function or significant variation is an interesting target with a comparatively small concentration variation in the population [90]. Similarly, albumin varies between 10% - 80% over a period of three days [1,66,85]. The clinical question is whether these moderate changes affect the diagnostic accuracy of the CRP test or systemic inflammation biomarker and the outcome of a clinical intervention. Considerable research is required to validate a quantitative correction of salivary CRP to predict accurately the serum, systemic biomarker concentrations.

If no correction function could be found of the evaluation time is significant then salivary CRP may still have several potential uses in detecting systemic inflammation as serum positive or negative. Quellet-Morin et al. and Out et al. both used salivary CRP levels to predict whether serum CRP levels exceeded a 3 mg/L cut-off to assess for cardiovascular disease. They produced results with sensitivities ranging from 66 to 89%, which are promising as 89% is considered a good measure of discrimination [33,39]. However, further research is required for using this cut-off to determine the tests specificities and true and false positive and negatives, which would be required for use in clinical practice. Lyengar et al. used salivary CRP to predict if serum levels exceeded alternative cut-offs of 5 and 10 mg/L with respective sensitivities and specificities of 64% and 94% for 5 mg/L and 54% and 95% for 10 mg/L [38]. The 10 mg/L cut-off is significant as CRP results at this value are clinically abnormal [91]. Other clinically relevant cut-off points could also be used. For example, 40 mg/L distinguishes between a bacterial and viral source of inflammation, consequently this cut-off value may help differentiate these inflammatory stimuli [28]. This would help to guide management and reduce the prescription of unnecessary antibiotics. The risk of using cut-offs when biomarkers are subject to differential dilution could lead to false negative results in screening.

Salivary CRP could also be applied alongside other markers to produce a composite score that indicates the presence of pathologies. For example, Floriano et al. used salivary CRP, myeloperoxidase, myoglobin and an electrocardiogram to detect acute myocardial infarctions with a sensitivity and specificity of 93% and 86%. This compares favourably to serum screening methods, but requires confirmation in larger cohort sizes where the diagnoses are unknown [92]. Nevertheless, despite further research being required, multiple potential uses of salivary CRP are available if a strong salivary-serum CRP relationship is not present.

Using salivary CRP to determine when serum CRP levels exceed cut-off points has clinical potential,. However, this use has significantly fewer applications and still requires considerable research, particularly in determining how localised trauma influences analyte levels and in developing a POC investigation tool. Therefore, the potential yield of research would be reduced, but if it can be used to accurately detect pathologies, then this may still provide a quick and accurate means of detecting systemic inflammation that is not currently possible.

6. Conclusion

Salivary CRP appears to be derived, presumably passively, from the serum, principally though the GCF as an exudate and then diluted ~10^4 in the mouth depending on stimulation, the patient response and critically on the collection protocol. Only with these major challenges solved can salivary CRP be a good quantitative predictor of systemic inflammation. It is currently a poor quantitative biomarker of systemic inflammation as it does not consistently and strongly correlate with serum levels. The effects of local inflammation could be overcome with a screening tool but a complex tool would be required with high

sensitivity and specify which would defeat the simplicity and utility of salivary sampling. Overcoming the variable dilution and local inflammation problems with a multiplexed biomarker panel could provide controls for both dilution and differential makers of local inflammation. Internal controls would recover the sampling simplicity but would require a significant validation process. In the interim a set of saliva positive – saliva negative tests may be nearer to clinical implementation of salivary testing. It would then need to compete effectively with urine analysis where similar problems of dilution, hydration and local *vs* system inflammation exist. The implementation of frequent systemic biomarker measurement in a non-invasive fluid remains attractive especially at point-of-care.

Conflict of interest declaration

Professor Shaw is a Director of Attomarker Ltd. which did not fund the research.

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