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ORIGINAL ARTICLE

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Investigating the utility of saliva immunoglobulins for the detection of myeloma and using myeloma proteins to clarify partition between oral and systemic immunity

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Abstract

Objectives: Myeloma is characterised by the presence of monoclonal immunoglobulin (M-protein) and the free light chain (FLC) in blood. We investigated whether these M-proteins and FLC are detectable in myeloma patients' saliva to evaluate its utility for non-invasive screening and monitoring of haematological malignancies.

Methods: A total of 57 patients with monoclonal gammopathy and 26 age-matched healthy participants provided paired serum and saliva samples for immunoglobulin characterisation and quantification.

Results: Myeloma patients had IgG or IgA M-protein levels ranging up to five times and FLC levels up to a thousand times normal levels of polyclonal immunoglobulins. Despite these highly elevated levels, only two IgG and no IgA M-proteins or FLC could be detected in paired saliva samples. Most patients had reduced levels of serum polyclonal immunoglobulins, but all had normal levels of salivary IgA.

Conclusions: Immunoglobulin transfer from blood is not determined by levels in the systemic circulation and more likely dictated by periodontal inflammation and the integrity of the oral epithelium. Immunoglobulins secreted by bone marrow plasma cells do not substantially enter saliva, which represents a poor medium for myeloma diagnosis. These findings, along with normal salivary IgA levels despite systemic immunoparesis, support a strong partitioning of oral from systemic humoral immunity.

KEYWORDS
haematological neoplasms, immunity, humoral, immunoglobulin light chains, immunoglobulins, multiple myeloma, paraproteins, plasma cells, saliva

NOVELTY STATEMENT: This provides the largest analysis of salivary immunoglobulins in monoclonal gammopathies to date. Monoclonal immunoglobulin secreted by neoplastic plasma cells in bone marrow can be distinguished from polyclonal immunoglobulin. We found that high levels of monoclonal immunoglobulins in blood did not transfer to saliva; consequently, saliva cannot be used to detect or monitor monoclonal gammopathies. Systemic and oral immunity are compartmentalised with little salivary immunoglobulin derived from blood; this has important implications for immunity to transmission of pathogens via the oral pharyngeal cavity.

1 | INTRODUCTION

Saliva is an attractive method of specimen collection that can offer several advantages over blood and tissue collection. It is non-invasive, collection requires no specialist training or equipment and may be more cost-effective. Consequently, saliva has received increasing interest as an alternative tool for the diagnosis of systemic diseases across various fields of medicine.[1,2] Salivary analysis may facilitate the early detection of malignancies and assist in monitoring treatment,[2] with salivary biomarkers associated with several different types of cancer.[3] However, the potential clinical value of saliva with regard to haematological malignancies has yet to be established.

Non-invasive screening is highly applicable to rapid immunodiagnostics and point-of-care testing. Many low-middle income countries do not have access to the laboratory tests required to investigate and diagnose myeloma. Measuring saliva κ:λ ratios on a rapid platform, such as lateral flow devices, could be a simple and low-cost way of detecting myeloma without the need for specialised laboratory tests. In addition, population-based screening for MGUS is currently being evaluated to improve myeloma outcomes by identifying those at high risk who may be suitable for early treatment.[4] A simple, cheap, non-invasive screening method without the need to send samples to a laboratory, such as a saliva lateral flow test, could be an effective way to support future large-scale MGUS or myeloma screening strategies. Accordingly, the utility of saliva in relation to monoclonal gammopathies warrants further investigation.

IgA is the most abundant antibody in saliva, followed by IgG. Most salivary IgA is dimeric and produced from plasma cells in the salivary glands.[5] A fraction (<20%) of salivary IgA is monomeric and thought to be serum derived.[6,7] Most salivary IgG was thought to be serum-derived through gingival crevicular fluid (GCF) via passive diffusion.[7–9] Consequently, the integrity of the epithelial barrier and oral health factors (including presence/extent of inflammation) influence the concentration of serum-derived antibodies in the saliva.[7,9] Indeed, periodontitis patients have been observed to have x5 IgG and x2 IgA concentrations compared with healthy individuals.[6]

Under normal conditions, there is no characteristic of salivary immunoglobulins that determines their origin from local or bone marrow plasma cells. Multiple myeloma (MM) is a cancer of Ig-producing plasma cells in the bone marrow diagnosed using serum biomarkers: monoclonal whole immunoglobulin (M-protein; 150 kDa) and κ or λ free light chains (FLC; 22.5 kDa). Plasma cells from MM patients secrete M-protein and FLCs unique to that neoplastic plasma cell clone, distinguishable from other immunoglobulins. Consequently, if these proteins are present in saliva, salivary analysis could offer a supportive tool in the detection of myeloma. Further, their levels in saliva provide a measure of the proportion of salivary immunoglobulins that are derived from blood.

Out of 3177 newly diagnosed MM patients, 95% had abnormal serum kappa: lambda FLC ratios, a third had FLC levels in excess of 100× normal and 75% had M-protein levels 2–20× normal serum immunoglobulin levels.[10] At such high systemic levels, we hypothesised that monoclonal proteins would be expressed in saliva via GCF, especially considering the median age of patients is 70 years: over 65-year-olds experience a 60% prevalence of periodontitis in the UK.[11] Oral inflammation has a positive association with low molecular weight serum proteins in GCF.[12] Older adults aged 60–80 years have significantly higher levels of FLCs in their saliva compared with young adults (<40 years old).[13] However, in healthy young adults, salivary FLC levels exhibit a diurnal variation, which is not displayed by serum FLC levels, suggesting salivary FLCs are predominately a product of local production.[14] Exploring salivary FLCs in MM with systemic FLC dysregulation would confirm any potential contribution of serum-derived FLCs to the oral environment. MM is characterised by immunoparesis with polyclonal immunoglobulins below normal in 85% of patients.[15] MM thus also enables the investigation of elevated M-protein levels and also suppressed polyclonal Igs concurrently within the same person.

The relationship between systemic and oral immunity still requires further understanding from both scientific and clinical perspectives. This has been highlighted recently by the COVID-19 pandemic where there may be separate systemic and mucosal immune responses to SARS-CoV-2 infection.[16] Due to the dual presence of both elevated monoclonal and suppressed polyclonal immunoglobulins in the systemic circulation, myeloma provides the perfect model to better appreciate interactions between mucosal and systemic systems.

There are several small studies of salivary Ig in MM from the 1970s.[17–19] However, salivary Ig in MM has not been further examined over the last 20 years despite the growing applications of salivary biomarkers and its advantages in disease populations and low-resource settings. More sensitive immunofixation techniques now exist and the advent of robust clinical assays to sensitively quantitate FLC opens the door to a thorough investigation of 1) the utility of salivary M-proteins and FLCs for detection of MM and 2) equally allow estimation of the contribution of serum-derived Igs and FLCs to total Ig levels in saliva.

2 | METHODS

2.1 | Study participants

Patients with myeloma or its precursor MGUS from outpatient clinics at the University Hospitals Birmingham and Royal United Hospitals Bath NHS Foundation Trusts participated. Blood samples (10mL) and saliva samples were taken at the same appointment. Healthy participants (HC cohort, $n = 26$) provided age-matched paired control serum and saliva samples. The study had ethical approval (NHS Research Ethics Committee, IRAS 238573 & 40073; University of

Birmingham Committee ERN_17-0213), and all patients gave written informed consent.

2.2 | Saliva sample collection and analyses

Whole saliva samples were collected by passive drooling for a timed 4-min period. Saliva volume was calculated assuming a saliva density of 1g/mL. Saliva flow rates (mL/min) were determined as volume÷collection time. Salivary FLC levels are below the calibration curves of commercial serum assays[13] and were quantified using ELISAs with a measurement range of 0.01–1mg/L (Abingdon Health Ltd).[20] ELISAs were used to quantitate salivary IgA (IBL International, Hamburg, Germany) and IgG (Abcam plc). Intra-assay coefficients of variation were <20% for salivary FLCs and <10% for IgG and IgA.

2.3 | Serological analyses and immunofixation electrophoresis

M-proteins were quantified and characterised using protein electrophoresis and densitometry (SPE; Interlab) and immunofixation (IFE, HYDRAS®). Serum FLC analysis (Freelite®, The Binding Site, UK) and IgG, IgA and IgM were measured on a Cobas® 6000 Modular (Roche Hitachi). A sub-cohort of MM patients were selected for paired serum and saliva IFE analysis on the basis of monoclonal serum protein above the detection limit (~100 mg/L) and representing M-protein levels from just above the normal range (NR) to highly elevated.

2.4 | Data analyses

Serology results were defined as within, below or above NR for immunoglobulins based upon 5th–95th centile ranges of adults aged ≥45 years in the UK (reported by Protein Reference Units): IgG 6–16g/L; IgA 0.8–4g/L; and IgM 0.5–2g/L. For serum FLC levels, the NRs were 3.3–19.4 mg/L for κ and 5.7–26.3 mg/L for λ and 0.26–1.65 for the sFLC ratio.[21–23]

Saliva flow rates affect immunoglobulin concentrations, and thus secretion rates (saliva flow rate x concentration) are important to reflect the total availability of protein at the oral mucosal surface and control for hydration status[24] and are reported alongside concentrations.

To compare Ig levels between MG and HC, Mann–Whitney *U*-tests were used. The Kruskal–Wallis test was used for 3 group analysis when MG was split based on κ or λ isotype. ROC curves evaluated saliva to detect elevated/suppressed levels of Ig in serum. All participants were analysed together to ensure sufficient sample sizes of those within/outside the NR. Accuracy was classified using AUC and associated sensitivity and specificity reported for best cut-offs. Spearman's rank correlation analysis was used to assess the relationship between serum and salivary parameters within the patient cohort.

3 | RESULTS

3.1 | Participant characteristics

The MG cohort (median age of 67 years; 63% male) had confirmed MM (n = 52), or MGUS (n = 5): IgG (61%), IgA (19%), IgM (4%), IgD (4%) or light chain only (LCO) myeloma (12%) and either κ (72%) or λ (28%) monoclonal FLC. Samples were associated with different stages of disease (diagnosis, undergoing therapy, remission and progression). Most patients (72%) serology results indicated active disease (elevated involved immunoglobulin and or FLC and abnormal ratio) with the remaining patients in remission. Of those with abnormal serology results, just over half (61%) were receiving therapy. There were no differences in salivary parameters based on active disease/remission or therapy/no therapy and patients with monoclonal gammopathy were analysed as one cohort herein. The HC cohort (42% male) had a median age of 69.5 years.

3.2 | Serum and saliva immunoglobulin and free light chain levels in myeloma patients vs healthy cohort

As shown in Figure 1, patients with monoclonal κFLC, monoclonal λFLC, IgG M-protein and IgA M-protein had significantly higher serum κFLC, λFLC, IgG or IgA, respectively, compared with the HC ($p < .001$ for all comparisons). For the FLC ratio, patients had a higher (κFLC patients, $p < .001$) or lower (λ FLC patients) ratio compared with the HC. There was no significant difference between salivary secretion rates of FLCs, FLC ratio IgG or IgA between MM and HC. The same was found for concentrations, with the exception of MM patients with a monoclonal κFLC isotype who had higher concentrations of salivary κFLC compared with the HC ($U = 366$, $p < .05$).

3.3 | Immunofixation of paired serum and saliva samples

Figure 2 contains immunofixation results from paired serum and saliva samples. IgG patients (1–7) had M-protein levels ranging from 51 g/L down to 4 g/L. Of these, 2/7 patients (1 and 4) had monoclonal IgG present in saliva. Patient 1 had a very large M-protein at 51 g/L and patient 4 a M-protein of 24 g/L. Patients 3 and 4 had the exact same M-protein level at 24 g/L, but only patient 4's IgG κ M-protein was detectable by IFE. Patient 2 with a sizable M-protein at 31 g/L did not have detectable M-protein in saliva.

Patients 8, 9 and 10 had IgA M-proteins of 63 mg/L, 21 mg/L and 6 mg/L respectively. Patients 8 and 10 had IgA visible, but the appearance was polyclonal and there was an absence of any clear monoclonal band.

Despite very high quantitation of monoclonal sFLCs in some IgG and IgA patients, including in excess of 2000 mg/L (e.g. patients 2 and 10), monoclonal FLCs were not visible in saliva. This was also the

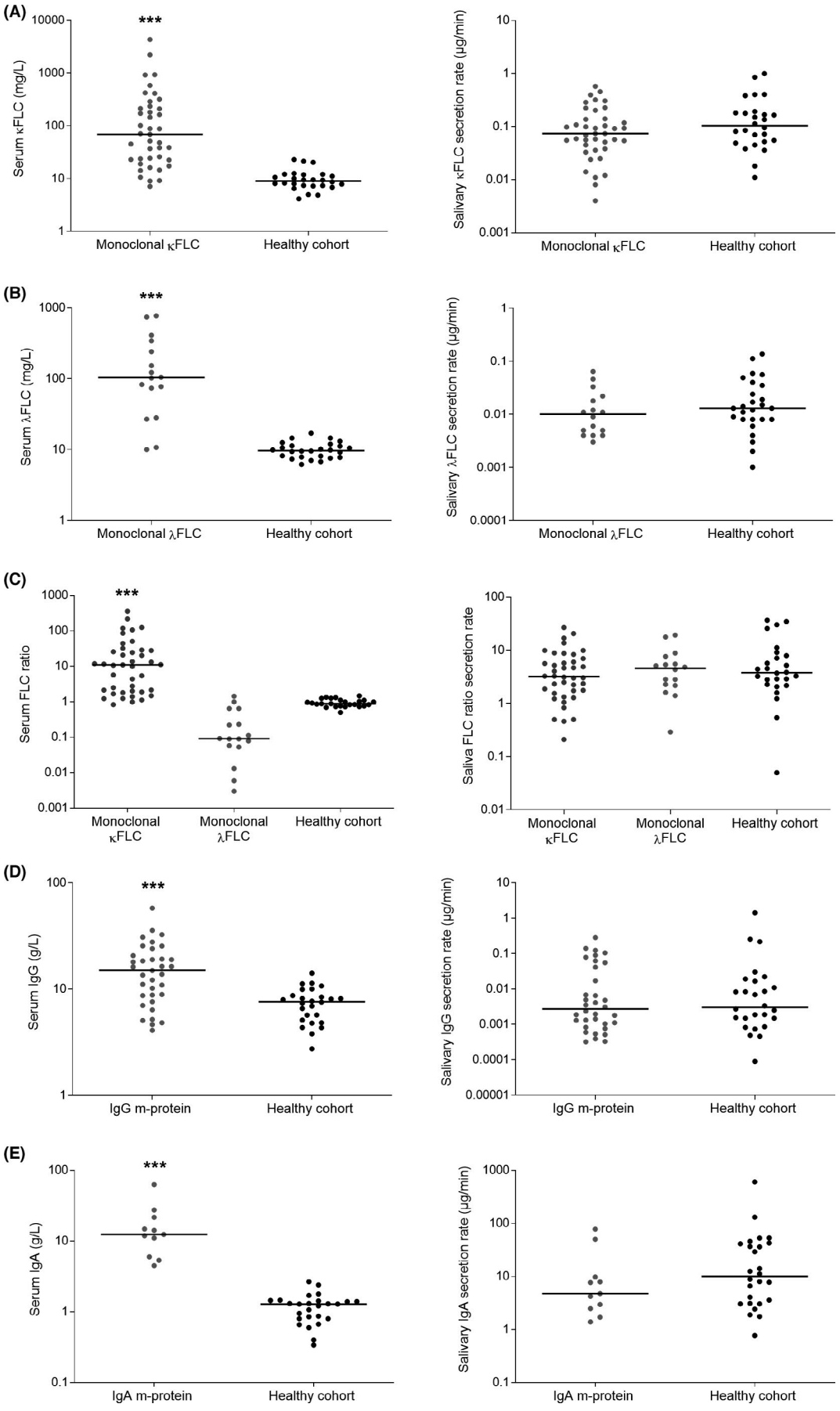

(A)
Serum κFLC (mg/L)
10000
1000
100
10
1
***
Monoclonal κFLC
Healthy cohort
Salivary κFLC secretion rate (μg/min)
10
1
0.1
0.01
0.001
Monoclonal κFLC
Healthy cohort
(B)
Serum λFLC (mg/L)
1000
100
10
1
***
Monoclonal λFLC
Healthy cohort
Salivary λFLC secretion rate (μg/min)
1
0.1
0.01
0.001
0.0001
Monoclonal λFLC
Healthy cohort
(C)
Serum FLC ratio
1000
100
10
1
0.1
0.01
0.001
***
Monoclonal κFLC
Monoclonal λFLC
Healthy cohort
Saliva FLC ratio secretion rate
100
10
1
0.1
0.01
Monoclonal κFLC
Monoclonal λFLC
Healthy cohort
(D)
Serum IgG (g/L)
100
10
1
***
IgG m-protein
Healthy cohort
Salivary IgG secretion rate (μg/min)
10
1
0.1
0.01
0.001
0.0001
0.00001
IgG m-protein
Healthy cohort
(E)
Serum IgA (g/L)
100
10
1
0.1
***
IgA m-protein
Healthy cohort
Salivary IgA secretion rate (μg/min)
1000
100
10
1
0.1
IgA m-protein
Healthy cohort

FIGURE 1 Serum and salivary immunoglobulins compared between the healthy cohort and monoclonal gammopathy patients based on diagnosis. Patients are separated based on monoclonal FLC isotype or M-protein (A = monoclonal κFLC ($n = 41$); B = monoclonal λFLC ($n = 16$); C = all patients divided by FLC isotype; D = IgG M-protein ($n = 35$); E = IgA M-protein ($n = 11$)), and data are presented for the equivalent FLC/immunoglobulin. Serum concentrations are shown on the left and corresponding salivary secretion rates are shown on the right. As expected in individuals with/without myeloma, serum parameters were higher (or also lower in the case of FLC ratio) in patients compared with the healthy cohort. Significance vs. healthy cohort is indicated: ***$p < .001$, for all comparisons, Mann–Whitney *U*-tests (2 group) or Kruskal–Wallis test (3 groups). These findings were not mirrored in saliva: There was no significant difference in κFLC, λFLC, IgG or IgA salivary secretion rates between healthy controls and patients with the equivalent monoclonal FLC/M-protein

case in LCO patients. Polyclonal IgA was visible in the majority (9/12) of saliva samples, detectable just below 10 mg/L.

3.4 | Saliva detection of elevated myeloma serum biomarkers

Overall, saliva concentration (but not secretion rates) returned significant findings for differentiating individuals based on serum reference ranges. Although AUC values were <.70, with 95% confidence intervals (CI) crossing below 0.50 at times, and sensitivity (SE)/specificity (SP) generally <70%. The strongest finding was for saliva concentration identifying IgG >NR; although the AUC (.73) and SE (75%) and SP (70%) were moderate. Full results are summarised below and detailed in Table 1.

A total of 81% of patients had an abnormal sFLC ratio at the time of sampling. The entire HC group presented with normal ratios. In distinguishing between those with an abnormal vs normal ratio, saliva concentration returned a significant AUC (.64, $p < .05$). Using an extended reference range of.01–7,[25] participants were then subdivided into those with the most extreme ratios (44% of patients outside of this range) vs all participants within this range. This yielded an AUC.64 for concentration, which was borderline significant ($p = .05$).

Elevated serum κFLC was seen for 60% of all patients; 76% of patients with a monoclonal FLC isotype had elevated serum κFLC. Saliva parameters were unable to differentiate between those with serum κFLC within or above the NR. As some individuals had only marginally elevated FLCs, participants were further investigated using cut-offs of serum κFLC 50 mg/L and 100 mg/L, which returned significant AUCs for saliva concentration.

For serum λFLC, 26% of patients were above the NR. Saliva was unable to distinguish between normal and elevated serum λFLC levels. Numbers of those with elevated λFLC were insufficient to subdivide into more extreme values.

Half of the patients with IgG myeloma had elevated IgG, with the remaining patients exhibiting normal/below normal levels post-therapy/during remission. This equated to 21% of the total study cohort with serum IgG values above the NR. Saliva concentrations returned significant AUCs for identifying participants above the NR for IgG. All patients with IgA myeloma had IgA above the NR, accounting for 13% of the total study cohort. Saliva was unable to accurately distinguish between individuals with serum IgA above the NR.

3.5 | Polyclonal salivary immunoglobulins in patients and patients with immunoparesis

Patients with non-IgG MG and non-IgA MG had normal salivary IgG and IgA levels, respectively, relative to the HC (Figure 3). Patients with immunoparesis were identified and salivary parameters were compared with the HC with serum levels within the NR. In patients without an IgG M-protein ($n = 22$), 55% were below the NR for IgG. When comparing the non-IgG patients with IgG immunoparesis to the HC, patients had significantly lower salivary IgG secretion rates ($U = 35$, $p < .05$) (Figure 3). There was no significant difference in salivary concentration. When analysing all study participants together on the basis of serum IgG below the NR or within/above NR, ROC analyses returned significant, albeit modest accuracy, AUC for saliva concentration (0.67, $p < .05$) but not secretion rate (Table 1).

In patients without an IgA M-protein ($n = 46$), 54% were below the NR for IgA. For non-IgA patients with IgA immunoparesis, salivary IgA secretion rate (Figure 3) and concentration were not significantly different with the HC. Analysing the cohort overall with ROC analyses, saliva IgA parameters were unable to differentiate between individuals below the NR for serum IgA from those who were not (Table 1).

3.6 | Correlation of serum and salivary levels within the patient cohort

For the MG cohort, serum IgG significantly correlated with salivary IgG concentration ($r_s = .49$, $p < .001$) and secretion rate ($r_s = .37$, $p < .01$). Serum IgA was associated with higher concentrations of saliva IgA ($r_s = .32$, $p < .01$) but not IgA secretion. Serum κFLC was not correlated with salivary concentration or secretion of κFLC. Serum λFLC correlated with saliva λFLC rates only ($r_s = .36$, $p < .01$) and not saliva λFLC concentration.

4 | DISCUSSION

The present investigation provides the largest analysis of salivary Ig in MG to date and advances existing knowledge on the relationship between systemic and oral immunity. Elevated systemic immunoglobulin levels are not reflected in saliva. The lack of raised salivary Ig levels in the MG cohort suggests that monoclonal

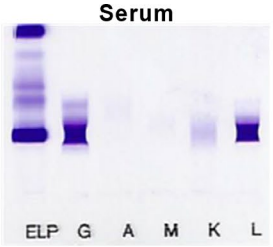
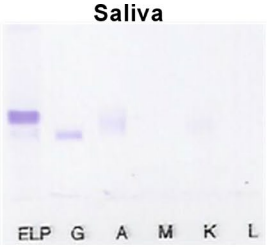
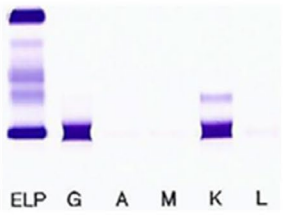
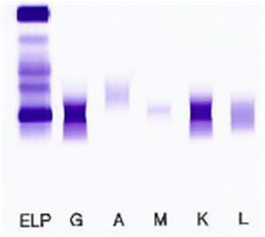
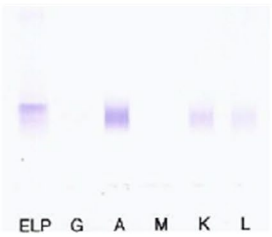
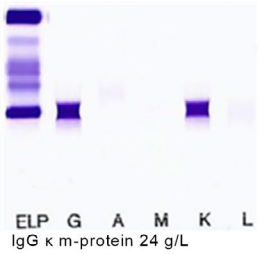
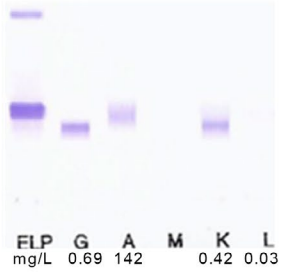
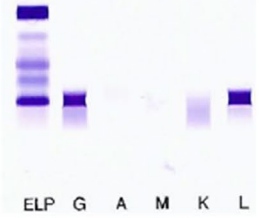
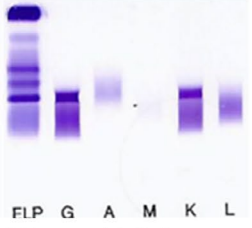
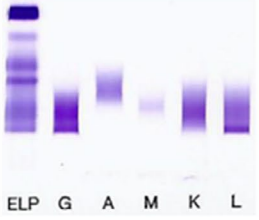
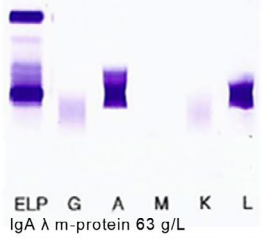
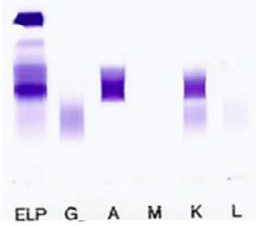
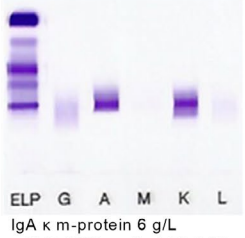
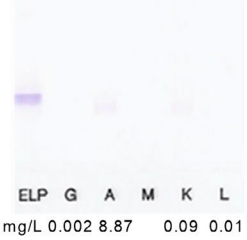
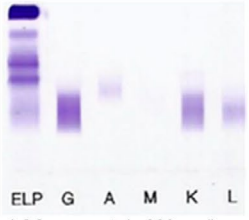
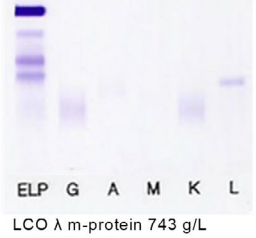
	Serum	Saliva	Serum	Saliva
1	 IgG λ m-protein 51 g/L κ FLC 9 mg/L; λ FLC 83 mg/L sFLCR 0.11	 mg/L 0.30 9.81 0.12 0.86 Monoclonal IgG band visible Polyclonal IgA κ	2  κ m-protein 31 g/L with monoclonal κ FLC 2219 mg/L λ FLC 10 mg/L sFLCR 222	 mg/L 0.02 8.63 0.13 0.02 Polyclonal IgA visible only
3	 IgG κ m-protein 24 g/L κ FLC 37 mg/L; λ FLC 15 mg/L sFLCR 2.47	 mg/L 0.02 22.48 0.06 0.07 Polyclonal IgA κ and IgA λ	4  IgG κ m-protein 24 g/L κ FLC 9 mg/L; λ FLC 6 mg/L sFLCR 1.5	 mg/L 0.69 142 0.42 0.03 Monoclonal IgG κ visible Polyclonal IgA visible
5	 IgG λ m-protein 15 g/L κ FLC 14 mg/L; λ FLC 10 mg/L sFLCR 1.4	 mg/L 0.21 108.0 0.87 0.02 Faint polyclonal IgG and IgA	6  IgG κ m-protein 8 g/L κ FLC 38 mg/L; λ FLC 9 mg/L sFLCR 4.2	 mg/L 0.01 10.88 0.07 0.02 No immunoglobulins visible
7	 IgG λ m-protein 4 g/L κ FLC 68 mg/L; λ FLC 105 mg/L sFLCR 0.65	 mg/L 0.01 75.7 0.38 0.05 Polyclonal IgA κ visible	8  IgA λ m-protein 63 g/L κ FLC 10 mg/L; λ FLC 774 mg/L sFLCR 0.01	 mg/L 0.002 10.22 0.37 0.03 Polyclonal IgA visible
9	 IgA κ m-protein 21 g/L κ FLC 102 mg/L; λ FLC 9 mg/L sFLCR 11.3	 mg/L 0.002 12.10 0.02 0.01 No immunoglobulins visible	10  IgA κ m-protein 6 g/L κ FLC 4308 mg/L; λ FLC 12 mg/L sFLCR 359	 mg/L 0.002 8.87 0.09 0.01 Faint polyclonal IgA visible
11	 LCO κ m-protein 289 mg/L λ FLC 7 mg/L sFLCR 4	 mg/L 0.22 0.03 No immunoglobulins visible	12  LCO λ m-protein 743 g/L κ FLC 4 mg/L sFLCR 186	 mg/L 0.33 0.07 Polyclonal IgA κ visible

FIGURE 2 Immunofixation of paired serum and saliva samples from myeloma patients. Data are shown for 12 patients with multiple myeloma: patients 1–7 have IgG M-protein and patients 8–10 have IgA M-protein: patients are ordered according to descending concentrations of M-protein. Patients 11 and 12 are light chain only (LCO) patients. Serum results are shown on the left and saliva on the right. M-protein concentration values reported were calculated using densitometry. Saliva IgA and IgA concentrations were obtained using ELISAs. Patients 11 and 12 did not have sufficient sample volume for IgA and IgG ELISA analysis

TABLE 1 Utility of saliva parameters to differentiate between individuals on the basis of reference ranges in serum

Serum parameters and ranges investigated	Saliva secretion AUC (95% CI)	Saliva concentration AUC (95% CI)
Outside or above normal range		
Serum FLC ratio NR </>(0.26–1.65)	0.43 (CI 0.30–0.56)	0.64 (CI 0.52–0.77)* Best cut-off 9.16 SE 65% & SP 61%
Serum FLC ratio </>extended NR (0.01–7.0)	0.50 (CI 0.36–0.64)	0.64 (CI 0.50–0.78)
κFLC >NR 19.4 mg/L	0.44 (CI 0.31–0.57)	0.54; (CI 0.41–0.67)
κFLC >50 mg/L	0.51 (CI 0.37–0.65)	0.64 (CI 0.50–0.79)* Best cut-off 0.34 mg/L SE 61% & SP 71%
κFLC >100 mg/L	0.56 (CI 0.40–0.72)	0.67 (CI 0.51–0.83)* Best cut-off 0.37 mg/L SE 59% & SP 75%
λFLC >NR 26.3 mg/L	0.53 (CI 0.37–0.69)	0.51 (CI 0.31–0.70)
IgG ≥NR 16 g/L	0.62 (CI 0.47–0.78)	0.73 (CI 0.61–0.86)** Best cut-off 0.0084 mg/L SE 75% & SP 70%
IgA ≥NR 4 g/L	0.45 (0.27–0.62)	0.59 (0.42–0.75)
Below normal range		
IgG <6 g/L	0.64 (0.50–0.78)	0.67 (0.53–0.81)* Best cut-off 0.0027 mg/L SE 78% & SP 58%
IgA <4 g/L	0.60 (0.46–0.74)	0.63 (0.50–0.76)

Note: Table reports area under curve (AUC) results with 95% confidence intervals (CI) in brackets. In the case of a significant AUC (*$p < .05$; ** $p < .01$), data are reported for best cut-off value and associated sensitivity (SE) and specificity (SP). Saliva secretion rates and concentrations were tested for their ability to identify individuals with serum immunoglobulin parameters above or below the normal range (NR). Analyses include all participant's ($n = 83$, 57 myeloma patients and 26 healthy cohorts).

proteins are either not transferred into the saliva of these patients or transferred at undetectable levels. The presence of high serum Ig itself does not favour increased transport into the saliva above what is seen in aged-matched individuals. This was confirmed by IFE where monoclonal protein and FLCs were not typically traceable in saliva.

These findings suggest transport of monoclonal proteins from serum to saliva is not characteristic of MG and independent of the amount of M-protein in the systemic circulation. Consequently, individual factors, such as oral health and periodontal inflammation and associated GCF flow, are likely to be the main determinant in serum protein transfer into saliva. Excessive serum protein production due to neoplastic plasma cells is likely only to be mirrored in saliva when the epithelial barrier is compromised.

The findings of the present investigation do not show concordance with all other studies that have included MM patients. Differences in the individual patient's studied, namely in their oral health, in addition to methodological differences/analytical techniques, likely contribute to mixed observations. A purely quantitative study found elevated serum Ig levels in MM patients was echoed in saliva.[17] Periodontal disease was excluded from the control group, and MM patients showed no signs of oral mucosal infection; however, signs of gingivitis or periodontitis were not explicitly described and are a major determinant of epithelial barrier integrity and serum ingress to saliva via GCF.[26] In addition, secretion rates were not examined to control for the impact of saliva flow on concentration, which can be influenced by medication usage and oral dryness arising due to health and lifestyle exposures including diabetes, smoking

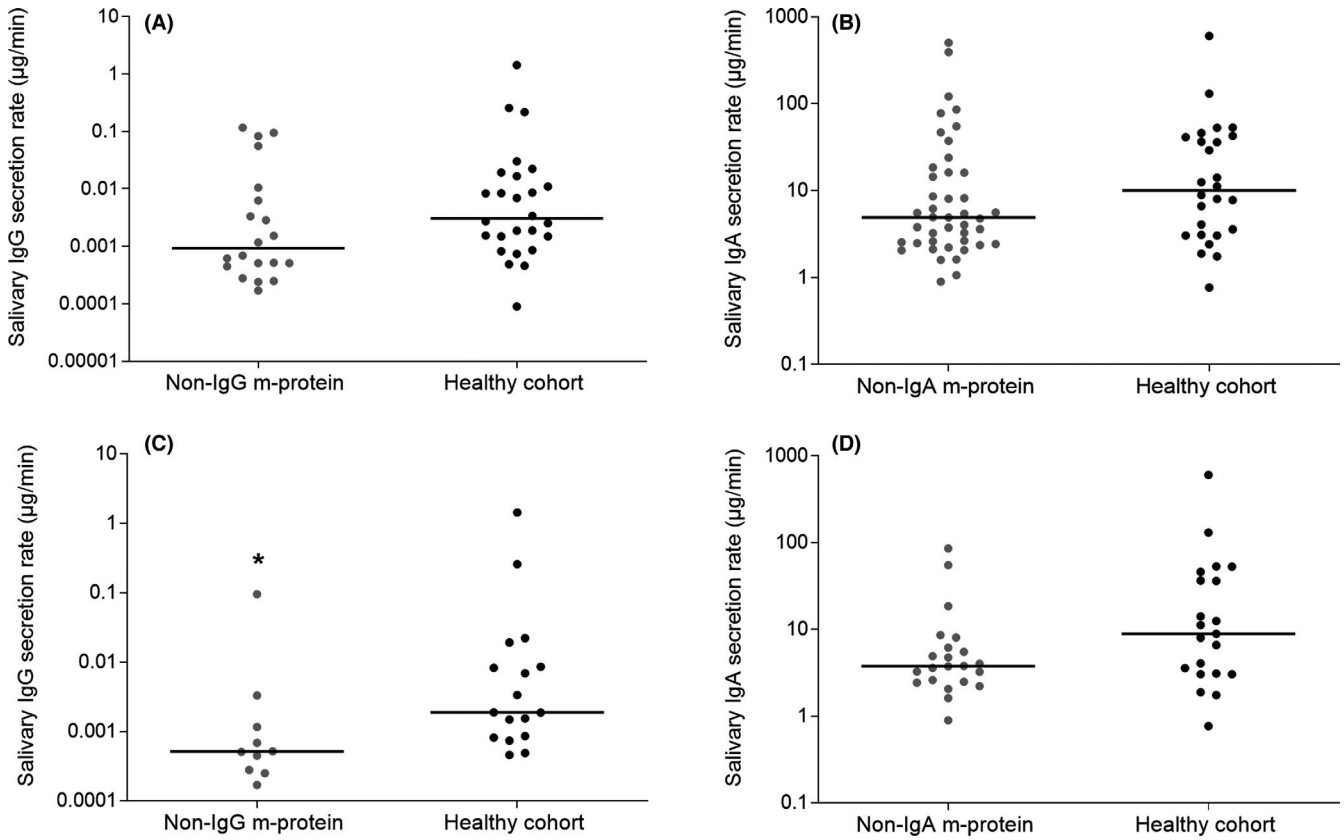


FIGURE 3 IgA and IgG salivary polyclonal immunoglobulin secretion rates in myeloma patients based on M-protein type compared with the healthy cohort. Data show salivary IgG secretion rates (A) and salivary IgA secretion rates (B) for all myeloma patients without an IgG M-protein, and without an IgA M-protein were not significantly different with healthy older adults. Panels C and D replicates the same analysis but for a sub-cohort of patients presenting with immunoparesis. Data are shown for salivary IgG secretion rates (C) and salivary IgA secretion rates (D) for myeloma patients without an IgG/IgA M-protein who demonstrated serum IgG/IgA levels below the normal range vs. healthy older adults with serum IgG levels within the normal range. $^{*}p < .05$ compared with the healthy cohort. Data were analysed using Mann–Whitney tests

and Sjörens syndrome.[27] Indeed, myeloma medication can affect saliva production, with sore/dry mouth reported in over a third of myeloma patients[28] and oral mucositis is a potential side effect of chemotherapy.

Coelho et al used electrophoresis to identify monoclonal IgA in five IgA M-protein patients and also monoclonal IgG in two IgG M-protein patients.[19] Findings suggested monoclonal heavy chain immunoglobulins, in the absence of FLCs, can be transferred to external secretions. This study recognised no overwhelming dominance of M-protein in the complex blend of proteins present in saliva. In our study, 2/3 IgA M-protein patients had visible polyclonal IgA in saliva; it cannot be discounted that monoclonal IgA may have been present but at a low concentration enabling it to be masked by polyclonal Ig. Mass spectrometry has emerged as a highly sensitive technique for diagnosing and monitoring multiple myeloma and detecting minimal residual disease.[29] It is now endorsed by The International Myeloma Working Group as an alternative for IFE.[30] A recent study showed the utility of mass spectrometry to detect low-level M-proteins in patients previously classified as non-secretory (immunofixation negative).[31] Mass spectrometry could facilitate establishing if monoclonal protein is detectable at low concentrations in saliva. However, although offering high analytical performance, this technique is still specialised and equipment costly compared with IFE.

As oral health status of patients was unknown, the transfer observed by Coelho et al should not be generalised as a feature of the disease per se. Brandtzaeg found an IgG M-protein patient with periodontitis to transmit higher than normal amount of IgG into saliva, but another IgG patient with a healthy mucosa had lower IgG compared with periodontitis patients without MM.[18] This suggests, in agreement with our findings, that high serum immunoglobulin levels do not inevitably mean higher levels in saliva and transmission is primarily determined by individual periodontal inflammatory status.[9,18] This is supported by the lack of correlation between immunoglobulins in serum and saliva seen in previous studies.[17,18] In the present study, serum IgG and IgA positively correlated with saliva concentrations, but the strength of association was weak.

To our knowledge, this study is the first to investigate saliva as a tool to distinguish abnormal Ig and FLC levels in serum. Saliva appears to be of limited value for this purpose. Analyses generally returned AUCs of limited accuracy. The best accuracy was observed for identifying IgG levels above normal. Although interesting from

academic perspective, the associated sensitivity and specificity (75% and 70% respectively) do not permit use in a clinical setting to identify elevated serum immunoglobulins. The use of saliva as a biomarker for identifying systemic disease has been recognised to be subject to periodontal health.[32] This appears to also be the case for MG. Other clinical conditions are associated with hypergammaglobulinemia, such as Sjogren's syndrome and IgG4-related disease[33–35]; studying the relationship between salivary and serum immunoglobins in such patients would be of interest.

The present investigation found that overall patients with MG had normal levels of salivary Igs, consistent with a previous study comparing myeloma to non-MM controls.[17] However, sub-group analysis found patients who specifically had IgG immunoparesis had lower salivary IgG secretion rates compared with the HC. ROC analyses also returned a significant result for saliva IgG concentration identifying those below the NR in serum. Although, again, findings were not strong enough to endorse the utility of saliva to identify IgG suppression in clinical practice. Further, saliva IgA could not differentiate on the basis of NR in serum.

Polyclonal IgA could be identified in the majority of salivary secretions measured using IFE. IgA is sourced mainly from local production, and recent evidence suggests FLCs in saliva are also locally produced.[14] The absence of monoclonal FLC in saliva and failure to detect a perturbed κ:λ FLC ratio in saliva adds further weight to this view, with malignancy itself not promoting transfer from serum. As a result of new sensitive quantitation,[20] salivary FLCs are an emerging biomarker in health research[13,14,36]; this increased understanding of their origin will inform future studies.

Findings demonstrate a strong partitioning between oral and systemic humoral immunity. This has important clinical implications and may be relevant to a broad range of other scientific and disease areas. Firstly, antibodies present in serum may not translate into local protection in the upper respiratory tract. This is an important consideration when determining patients' protection against disease and vaccination efficacy and warrants further investigation, particularly in blood cancers associated with immunosuppression and morbidity and mortality from infection. Secondly, our finding of division of immunity echoes emerging understanding from the COVID-19 pandemic. Research has demonstrated distinct systemic and nasopharyngeal immune responses to SARS-CoV-2 infection.[16] Indeed, mRNA vaccination against COVID-19 elicits strong systemic IgG immune responses but do not translate into mucosal immune activation; highlighting the need for effective mucosal vaccines against pathogens that primarily enter via oral and mucosal surfaces.[37–39] The present study adds to the growing scientific discussion of separation of immunity.

A limitation of the present study was that we were unable to carry out periodontal examinations on patients. This would have better enabled the isolated cases of M-protein transfer from serum to saliva to be conclusively linked to oral inflammation. The HC was of a similar age to the MM patients and while it would be expected that they would have similar oral health status, a dental examination would be needed to provide certainty.

In conclusion, despite high levels of M-proteins in serum these are not usually identifiable in saliva using quantitation or immunofixation. In a few individuals, M-proteins were identified in saliva, and this is likely determined by oral inflammation and integrity of the epithelium and was unrelated to serum M-protein level. The lower molecular weight FLC can be up to 1000-fold normal levels in serum and yet paired saliva FLC levels are normal. Saliva is therefore of no clinical use to detect/monitor M-protein or FLC production in monoclonal gammopathies. Results indicate that salivary immunoglobulin and FLC are almost exclusively derived from local plasma cells. Findings suggest that oral and systemic immunity should be viewed as separate compartments.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

DATA AVAILABILITY STATEMENT

Data in this manuscript concern patient data and are not available in a public repository. Data from the laboratory analysis included in this study are available on request.

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