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Saliva-omics in plasma cell disorders- Proof of concept and potential as a non-invasive tool for monitoring disease burden

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A B S T R A C T

Multiple Myeloma (MM), the second most common lymphoid cancer worldwide, is characterised by the uninhibited proliferation of terminally differentiated B-lymphocytes. Leading to The diagnosis typically requires the presence of a monoclonal protein (M protein) and the demonstration of CRAB features (hypercalcemia, renal impairment, anaemia and bone lesions). MM is considered incurable as, due to serial clonal evolution, the vast majority of patients succumb to treatment-refractory disease. MGUS (Monoclonal Gammopathy of Unknown Uncertain Significance) is the pre-malignant form of MM and, although 93% of MM patients exhibit M protein production associated with MGUS before diagnosis, little is known about the switch from pre-malignant to malignant disease. To explore this disease transition further, LC-MS/MS analysis was carried out to identify potential salivary biomarkers to monitor disease burden. FABP5 was detected in saliva as having a significant increase in abundance when MGUS was compared to symptomatic MM. The levels of FABP5 decreased after treatment indicating correlation with tumour burden. This finding was validated using western blot analysis and ELISA analysis.

Significance: The field of biomarker discovery has focused largely on serum as a biofluid. Saliva is a readily available biofluid that, as a biomarker resource, has been relatively un-explored. The identification of changes in saliva indicating disease progression underlines the utility of saliva as a non-invasive source of informative biomarkers reflecting disease burden and progression.

1. Introduction

With multiple verified biomarkers for MM, very little research has been conducted in the area of salivary biomarkers for the disease. It has been observed that approximately 40% of cancer, stroke and cardiovascular disease biomarkers are present in whole saliva [1]. Due to the invasiveness of serum collection, saliva biomarkers seem to be the logical progression in disease detection and diagnosis. Salivary biomarkers have revealed significant promise in the area of cancer detection over recent years.

In a study carried out in 2009 it was noted that CA15-3 levels, in both serum and saliva, was significantly increased in stage 2 breast cancer patients [2]. This was evidence to begin to establish CA15-3 as a salivary biomarker for breast cancer, along with the 65% detection in saliva of CA15-3 in breast cancer [3] and a 62% sensitivity observed. Epidermal growth factor (EGF) has been noted as being significantly higher in the saliva of women with primary or recurrent breast cancer in comparison with healthy controls. The most significant increased expression was noted in the saliva of women with local recurrence [4]. As this protein has been seen to play an important role in tumorigenesis, invasiveness and is known to be responsible for a variety of tissue growth and repair associated with poor prognosis, EGF is seen to be a potential salivary biomarker for breast cancer, especially since therapeutic target pharmaceuticals have already been approved by the FDA in the treatment of multiple cancer types [5]. This increased expression of EGF, along with increased vascular endothelial growth factor (VEGF) and carcinoembryonic antigen (CEA), was more recently observed in 2008 [6].

Saliva has proven itself useful in the detection and diagnosis of oral cancer (OC), a malignancy referring to the oral cavity, lip and pharynx. The majority of oral cancers are referred to as oral squamous cell carcinoma (OSCC). Interleukins such as IL-6, IL-8 and IL-1β have shown a significant increase in the saliva of OC patients in comparison with healthy controls. IL-8, specifically, has shown great promise in the search for early detection biomarkers in saliva for OC [7]. Metalloproteinases (MMP-1, MMP-3, MMP-10, MMP-12) have been commonly associated with multiple cancer types, including OSCC, in recent years and are thought to play a role in metastases and tumour invasion [8]. The over expression of MMP-1 and MMP-3 have been noted as over expressed in OSCC patients in comparison to cancer free control patients, with an observed trend towards higher expression with increasing disease severity [9]. A 75% increase in the expression of MMP-2 was observed [10] in the saliva of OSCC patients in comparison to healthy controls.

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A pilot study, carried out by Katz and colleagues observed that increased levels of salivary AGEs (advanced glycation endproducts) may act as a good way to determine biomarkers regarding the development of bone lesions in MM patients, especially those who have decreased marker expression for the progression of bone lesions. It was noted that patients who show multiple bone lesions also exhibit a significantly higher concentration of AGEs in both plasma [11] and saliva [12]. AGEs are proteins that are post-translationally modified and are known to act as markers of oxidative stress. They have been previously implicated in the proliferation of multiple types of cancer, such as prostate, OSCC, brain, breast and ovarian cancer by triggering proliferation, angiogenesis and inflammatory reactions during cancer progression [13]. These findings show great promise in the use of salivary biomarkers for disease diagnosis and bone lesion formation in MM.

Salivaomics has become an area of great interest in disease diagnosis over the last number of years, following the footsteps of the other "omics" based diagnostic tools. Saliva has been referred to as "the mirror of the body" as it gives an insight into the internal pathological state [14]. As saliva is considered a fast, inexpensive and non-invasive method of sample collection, the future of diagnosis, early detection, monitoring and prediction of progression of disease has been thought to lie here. Unfortunately, the identification of saliva biomarkers has taken time and more research is still required for the clinical use of these biomarkers.

2. Materials and methods

2.1. Patients and samples

The ethics committees of the participating hospitals approved the study in compliance with the Declaration of Helsinki. A total of 91 saliva samples were collected from patients at varying diagnosis and at varying treatment stages (Supplementary Table.1). Of the 91 saliva samples collected, a cohort of 38 samples was analysed by LC-MS/MS for in-depth discovery analysis of disease progression (Supplementary Table. 2). Saliva sample collection was carried out using the GBO Saliva Collection System, requiring patients to thoroughly rinse the oral cavity for 2 min using the saliva extraction solution. The solution was then collected into sterile collection tubes and stored at −80 °C.

2.2. Label-free LC-MS/MS analysis of patient saliva samples

Prior to mass spectrometric analysis samples were purified by acetone precipitation. 5 times the sample volume of cold 100% acetone was added to each sample and stored overnight at −20 °C. Samples were centrifuged at 15,000 × *g* for 15 min at 4 °C. The supernatant was decanted, and samples centrifuged again at 15,000 × *g* for 5 min. The supernatant was discarded, excess supernatant was removed and the resulting pellet was allowed to air-dry for 10 min. The pellets were re-suspended an appropriate volume of label-free solubilisation buffer and vortexed and sonicated to ensure full re-suspension. The protein amount was estimated using an RC/DC protein assay from Bio-Rad. BSA was used as a standard. Protein concentrations were equalised with label-free solubilisation buffer (6 M urea, 2 M thiourea, 10 mM Tris, pH 8.0 in LC-MS grade water) and 30 μg of protein was processed by the filter aided sample preparation (FASP) method (Wiśniewski et al., 2009) using a trypsin to protein ratio of 1:25 (protease: protein). Following overnight digestion and elution of peptides from the spin filter, 2% TFA in 20% ACN was added to the filtrates (3:1 (*v*/v) dilution). An Ultimate 3000 NanoLC system (Dionex Corporation, Sunnyvale, CA, USA) coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific) in the Proteomics Suite at Maynooth University was used for mass spectrometry-based analysis. Three technical replicates were analysed using label-free LC-MS/MS per patient sample. *Re*-suspended peptide mixtures (a maximum load of the equivalent 1 μg pre-digested protein) were loaded by an autosampler onto a C18 trap column (C18 PepMap, 300 μm id × 5 mm, 5 μm particle size, 100 Å pore size; Thermo Fisher Scientific). The trap column was switched on-line with an analytical Biobasic C18 Picofrit column (C18 PepMap, 75 μm id × 500 mm, 2 μm particle size, 100 Å pore size; Dionex). The peptides generated were eluted over either 65 min or 180 min using the following binary gradients: solvent A [2% (*v*/v) ACN and 0.1% (v/v) formic acid in LC-MS grade water] and 0–90% solvent B [80% (v/v) ACN and 0.1% (v/v) formic acid in LCMS grade water]. The column flow rate was set to between 0.25 and 0.3 μL/min [15,16]. The Q-Exactive was operated in positive, data dependent mode and was externally calibrated. Survey MS scans were conducted in the 300–1700 *m/z* range with a resolution of 140,000 (m/z 200) and lock mass set to 445.12003. CID (collision-induced dissociation) fragmentation was carried out with the fifteen most intense ions per scan and at a resolution of 17,500. A dynamic exclusion window was applied within 30 s. An isolation window of 2 *m/z* and one micro-scan were used to collect suitable tandem mass spectra.

2.3. Data analysis of all statistically significant proteins with altered abundance

Protein identification and label-free quantification (LFQ) normalisation of MS/MS data was performed using MaxQuant v1.5.2.8 (http://www.maxquant.org). The Andromeda search algorithm incorporated in the MaxQuant software was used to correlate MS/MS data against the *Homo sapiens* Uniprot reference proteome database and a contaminant sequence set provided by MaxQuant. Perseus v.1.5.6.0 (www.maxquant.org/) was used for data analysis, processing and visualisation. Normalised LFQ intensity values were used as the quantitative measurement of protein abundance for subsequent analysis. The data matrix was first filtered for the removal of contaminants and peptides identified by site. LFQ intensity values were log2 transformed and each sample was assigned to its corresponding group. ANOVA-based multi-sample *t*-test were performed using a cut-off of $p < 0.05$ on the post imputated dataset to identify statistically significant differentially abundant proteins. Receiver-operating characteristic (ROC) curve analysis was performed as it is a useful tool in assessment of biomarker accuracy. The ROC plots were obtained by plotting all sensitivity values (true positive fraction) on the y-axis against their equivalent (100-specificity) values (false positive fraction) for all available thresholds on the x-axis (MedCalc for Windows 8.1.1.0, Medcalc Software, Mariakerke, Belgium). The area under the curve (AUC) was calculated to provide a summary of overall classifier effectiveness. In our study, we consider AUC values ranging from 0.5 → 0.7 as poor, 0.7 → 0.8 as average, 0.8 → 0.9 as good and > 0.9 as outstanding.

2.4. ELISA for validation of decreased abundance of FABP5 from newly diagnosed MM to remission

50 μL of crude saliva and serum samples were added to antibody-coated microtiter wells and incubated at room temperature for 2 h, as directed by the manufacturers' recommendations. All manufacturers guidelines were followed, unaltered (AssayPro, USA).

2.5. Immunoblotting for validation of increased abundance of FABP5 throughout disease progression

20 μg of acetone precipitated protein, quantified using a Bradford assay, from MGUS and newly diagnosed MM patient samples were loaded into each lane and an SDS-PAGE gel was run. Immunoblot analysis were prepared in 2 × standard Laemmli-type buffer for one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [17], heated at 97 °C for 7 min and loaded onto hand-cast 10% SDS-PAGE gels. Proteins were transferred to nitrocellulose membranes, blocked in a milk protein solution (2.5% (*w/v*) fat-free milk powder in 10% phosphate-buffered saline and incubated overnight in

1:1000 diluted primary antibody. Following a number of washing steps, membranes were incubated with peroxidase-conjugated secondary antibodies, and immuno-decorated protein bands visualized using G:BOX Chemi XRQ (Syngene). Densitometric analysis of each blot was carried out using ImageJ software (NIH, Bethesda, MD, USA), along with Graph-Pad Prism software (San Diego, CA, USA) and BioRender.com, in which statistical significance was based on a p value ≤ 0.05. For coomassie staining loading controls, proteins were run on 10% SDS gels and incubated in fixing solution (50% methanol, 10% glacial acetic acid) for 1 h with gentle shaking. Gels were then incubated in staining solution (0.1% Coomassie Brilliant Blue R-250, 50% methanol, 10% glacial acetic acid) for 20 min, followed by incubation in de-staining solution (40% methanol, 10% glacial acetic acid) solution. This solution was renewed 3 times before exposure of gels using the G:BOX Chemi XRQ (Syngene).

3. Results

3.1. Comparative LC-MS/MS analysis carried out on saliva samples from pre-malignant MGUS to malignant newly diagnosed MM

To determine and examine the change in proteomic signatures of 8 MGUS and 18 newly diagnosed MM saliva samples, label free LC-MS/MS analysis was carried out. 152 proteins with altered abundance were positively identified. 6 of these proteins were identified as being statistically significant ($p < 0.05$), all 6 were observed to have an increased abundance in newly diagnosed MM in comparison to MGUS saliva samples (Table 1). Of the proteins determined to be statistically significant, Fatty Acid Binding Protein 5 (FABP5) was identified as having the highest fold change from MGUS to newly diagnosed MM and was found to have the highest AUC value compared to the other significant proteins - Glucose-regulated protein (AUC = 0.778; $p = 0.0029$); Xanthine Dehydrogenase (AUC = 0.813; $p = 0.0003$); Transgelin-2 (AUC = 0.771; $p = 0.0044$); FABP5 (AUC = 0.826; $p = 0.0001$); Cystatin-C (AUC = 0.747; $p = 0.0171$); Calmodulin-like protein (AUC = 0.764; $p = 0.0085$).

3.2. Comparative immunoblotting analysis of increased abundance of FABP5 for MGUS verses newly diagnosed MM

To independently verify the increased abundance of FABP5 observed in newly diagnosed MM in comparison to MGUS, determined by comparative proteomics, immunoblotting was carried out with focus on epidermal fatty acid binding protein (FABP5). Immunoblotting of 4 MGUS saliva samples and 4 newly diagnosed MM samples identified a clear increased abundance of FABP5 in newly diagnosed MM compared to MGUS (Fig. 1).

3.3. Area under the curve (AUC) receiver operating characteristic curve (ROC) analysis of increased abundance of FABP5 for MGUS verses newly diagnosed MM

The AUC ROC value for FABP5 was calculated in this study. The AUC was found to have good discriminatory power for FABP5 as a potential biomarker for the change from MGUS to newly diagnosed MM, with a value of 0.826 (Fig. 2), according to guidelines published by Hosmer & Lemeshow [18]. With a p-value of 0.0001 ($p < 0.05$), 100% sensitivity value and specificity value of 66.67%, these values represent very strong discriminatory power for the identification of a change from MGUS to MM.

3.4. ELISA analysis of FABP5 abundance throughout disease progression of saliva samples at varying time points

ELISA analysis was carried out on crude saliva samples outlined in Table 2, identifying an increased abundance of FABP5 throughout disease progression. Three of the five patients included in the analysis showed a decreased abundance of FABP5 from newly diagnosed MM to remission (Fig. 3A). A decreased abundance of FABP5 was also identified from newly diagnosed MM to post Bortezomib, Melphalan and Prednisone (VMP) treatment (Fig. 3B). One of the five patients included in the analysis exhibited an increased abundance of FABP5 from newly diagnosed MM to remission (Fig. 3A). As ELISA analysis is utilised in clinical laboratories routinely, validation of FABP5 using ELISA analysis was carried out to show transferability to a clinical setting.

3.5. Comparative LC-MS/MS analysis carried out on saliva samples from patients at multiple time points

In-depth proteomic analysis of saliva samples from 7 patients, 6 of which had two time points and 1 of which had three time points, was carried out using label-free LC-MS/MS, to determine the change in proteomic signature in individual patients at differing time points throughout disease progression, ranging from MGUS, newly diagnosed MM, post treatment and remission (Table 2). 74 statistically significant proteins were identified as having a common altered abundance across 15 saliva samples analysed (Supplementary Table 3). As patient 7 had samples taken at three different time points, comparative analysis was carried out as newly diagnosed MM versus partial response to treatment, partial response to treatment versus remission and newly diagnosed MM versus remission. Of the 74 statistically significant proteins identified, Protein-glutamine gamma-glutamyltransferase E/ Transglutaminase (TGM3) was identified as having the most consistent trend across all patient samples examined, with 6 of the 9 comparisons exhibiting a decrease in abundance of TGM3 from unfavourable to more favourable conditions (Table 3). 2 of the 9 samples analysed did not indicate the presence of TGM3. Interestingly, Beta-2-microglobulin (β2M), a well established biomarker for MM, was identified in the saliva samples after mass spectrometric analysis (Table 3). The clinical significance of the identification of β2M further validates the potential use of saliva as a biofluid for diagnosis.

4. Discussion

Serum biomarkers have become an important tool in the diagnosis of multiple myeloma, increasing the criteria for diagnosis to include three vital biomarkers for the disease [19]. All verified biomarkers for MM are, however, serum biomarkers. The collection of serum is a

Table 1
Proteins Identified by Label-Free LC-MS/MS with a Statistically Significant Altered Abundance from MGUS to Newly Diagnosed MM.

Accession number	Protein name	Fold change	p-value	No. unique peptides	Coverage (%)
P01034	Cystatin C	1.89	0.043	4	30.8
P11021	78 kDa Glucose-Regulated Protein	3.44	0.023	18	35.9
P27482	Calmodulin-like Protein 3	3.31	0.021	8	63.1
P37802	Transgelin-2	5.22	0.047	6	42.2
P47989	Xanthine Dehydrogenase/Oxidase	1.59	0.033	5	3.8
Q01469	Fatty Acid-Binding Protein, Epidermal	35.25	0.0019	8	65.2

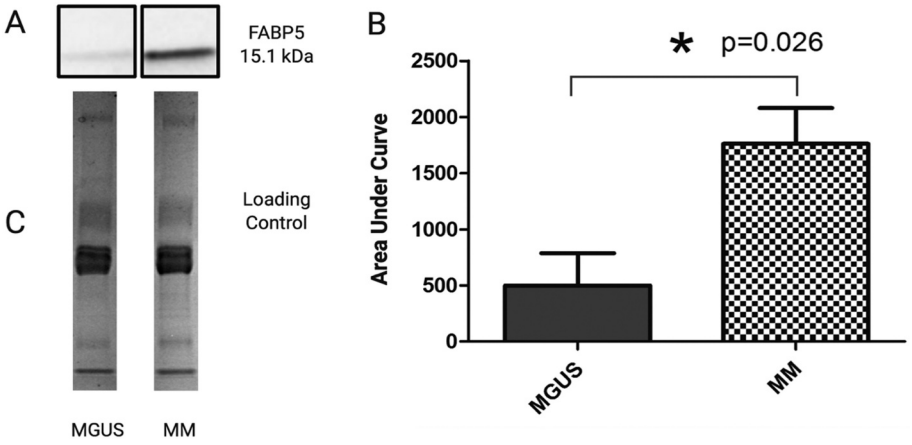
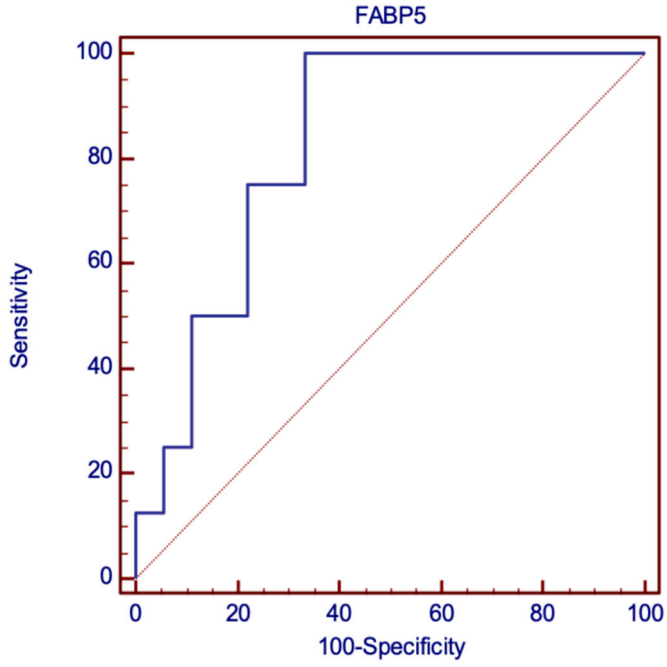


Fig. 1. Comparative Immunoblot Analysis of FABP5 Abundance from MGUS to Newly Diagnosed MM.
Shown is a representative immunoblot with immunodecorated bands labelled with an antibody specific to FABP5 (A). Lane 1 represent MGUS patient saliva and lane 2 represent newly diagnosed MM patient saliva sample. 20 μg of protein was loaded per lane. Bands quantified using ImageJ. B is the graphical analysis of the immuno-decoration (MGUS $n = 4$, MM n = 4) which was statistically evaluated using an unpaired Student's t-test, visualized using GraphPad Prism software (Mean values ± SEM; $n = 8$; *p < 0.05; **$p < 0.01$; ***$p < 0.001$). C depicts Coomassie stained gel to exhibit equal loading of samples in immunoblot with 20 μg of protein was loaded per lane.



Area under the ROC curve (AUC)	0.826
Significance level P (Area=0.5)	0.0001
Sensitivity	100
Specificity	66.67

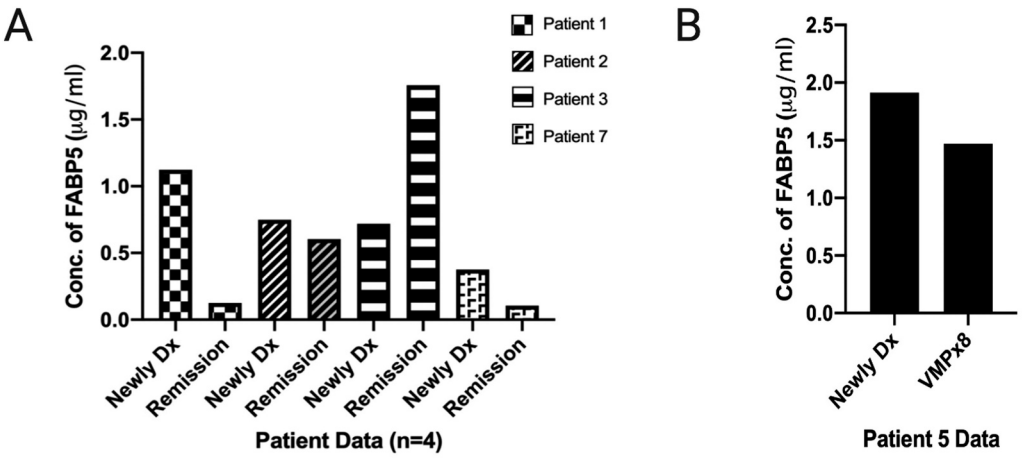
Fig. 2. Area Under the Curve (AUC) Receiver Operating characteristic Curve (ROC) analysis for FABP5 in MGUS versus MM.
AUC ROC analysis depicts the sensitivity and specificity of FABP5 as a potential biomarker for a switch between premalignant MGUS to malignant MM.

minimally invasive process, which has been noted to cause varying levels of stress/discomfort to patients. The progression in the search for new biomarkers is to consider saliva as a biofluid for analysis as saliva collection is a non-invasive process, it is inexpensive and a fast biofluid to collect. Limited research has been published, to date, based on salivaomics in relation to cancer biomarkers in general, especially in MM.

Fatty Acid Binding Protein 5 (FABP5) is one of several isoforms of FABP [20] and is known to enhance the transcriptional activity of nuclear receptor peroxisome proliferator-activated receptor β/δ [21] and promotes cell proliferation, survival and migration [22]. FABP5 has been observed to be implicated in the proliferation of cancer and has been seen to be increased in abundance in multiple cancer types such as breast [23], prostate [24], cervical [25] and HCC [26]. Interestingly, in a study carried out by [27] FABP5 in MM patients was associated with poor outcome and unfavourable clinical parameters. FABP5 has been observed, in this study, to have a statistically significant increase between MGUS and MM. The abundance of FABP5 is significantly higher ($P < 0.002$) in the newly diagnosed MM patient samples in comparison with the MGUS patient samples (Table 3). This would lead us to believe that FABP5 levels increase as the disease progresses. AUC ROC analysis also alludes to the potential of FABP5 as a potential salivary biomarker from MGUS to MM, with an AUC value of 0.826, sensitivity of 100% and statistical significance of $p = 0.0001$ (Fig. 2). Based on the data in this study, FABP5 is predicted to be a useful salivary marker in the determination of disease progression as this information correlates with multiple distinct types of cancer. Comparative immunoblot analysis further confirmed the increased abundance of FABP5 from premalignant MGUS to newly diagnosed, malignant MM (Fig. 1). Although a lack of overlap with the altered abundance of FABP5 in saliva samples from patients at multiple time points and MGUS vs newly diagnosed MM was identified using LC-MS/MS, ELISA analysis identified a decrease in the abundance of FABP5 in serial samples from newly diagnosed to remission in four of the five patients tested (Fig. 3). ELISA analysis was preformed to identify an increase in abundance of FABP5 as this technique is commonly used in clinical laboratories to measure for biomarker abundance and could aid a smooth transition into clinical

Table 2
Patient Details for Saliva Samples used in Time Point Analysis including date of sample collection.

Patient ID	1st sample	2nd sample	3rd sample
Pt.1	Newly Diagnosed MM (March 2013)	Remission (January 2014)	
Pt. 2	Newly Diagnosed MM (March 2013)	Remission (June 2013)	
Pt. 3	Newly Diagnosed MM (March 2013)	Remission (May 2013)	
Pt. 4	Post Treatment (January 2013)	Relapse (February 2014)	
Pt.5	Newly Diagnosed MM (January 2013)	Post Treatment (VMPx8) (April 2013)	
Pt. 6	Remission (January 2013)	Post ASCT Transplant (September 2013)	
Pt. 7	Newly Diagnosed MM (December 2012)	Partial Response (April 2013)	Remission (October 2013)



C

Accession Number	Protein Name	Patient 1	Patient 2	Patient 3	Patient 7	Patient 5
Q01469	Fatty Acid-Binding Protein, Epidermal	↓	↓	↑	↓	↓

Fig. 3. Bar Chart of ELISA Analysis Comparing Abundance of FABP5 in Saliva of Serial Sample Patients. A depicts a comparative bar chart of the change in abundance of FABP5 in four of five serial saliva patients from newly diagnosed MM and remission (Table 2). B depicts a comparative bar chart of the change of abundance in one patient from newly diagnosed MM to post 8 cycles of treatment with Bortezomib, Melphalan and Prednisone (VMP). Analysis was carried out using ELISA analysis. Each colour represents an individual patient at each of two time points. A decreased abundance of FABP5 can be observed in three of the four patients from newly diagnosed MM in comparison to remission saliva samples (A). A decreased abundance of FABP5 can also be observed after 8 cycles of treatment with VMP (B). 50 µL crude saliva was analysed per sample. C depicts the trends that are observed in the abundance of FABP5 in serial samples, increased abundance shown as ↑ and decrease abundance shown as ↓. Details of patient saliva samples used for ELISA analysis are outlined in Table 2.

utility in the future. This, again, leads to the prediction that an increase in FABP5 is directly linked to disease progression and severity.

β2M (Beta-2-microglobulin) was significantly decreased in abundance in three in seven patients from diagnosis to remission (Table 3). β2M, in combination with albumin, has been established, since 2005, as a predictive biomarker for disease progression and stage according to the ISS. The ISS uses serum β2M to provide three stage classifications with three different median survival periods, establishing that lower abundance of β2M is directly correlated with increased overall survival [28]. The significant decreased abundance noted in this study further supports the finding of the ISS (the increased expression of β2M indicates decreased overall survival) and strengthens the use of saliva as a biofluid for prediction of disease progression as it mirrors the findings in serum. β2M is a widely known housekeeping gene and interacts and stabilizes "tertiary structures of the major histocompatibility complex class I α-chain for presenting antigenic peptides from intracellular proteins to cytotoxic T lymphocytes" [29]. In a study carried out by [30] the significant increased abundance of β2M in non-responders in comparison to responders to thalidomide based therapy was recorded providing a link to over expression to β2M and drug resistant in multiple myeloma. This β2M increase in drug resistance has also been link to resistance using bortezomib for treatment of MM [31]. The presence of β2M, with increased abundance, in the saliva of MM patients again solidifies the relevance of proteomic analysis on patient saliva samples for predictive markers for disease progression.

An increased abundance of multiple proteins during disease progression at different time points has been noted, and specifically a significant decreased abundance from newly diagnosed to remission in patient samples. Protein-glutamine gamma-glutamyltransferase E or Tranglutaminase-3 (TGM3) was seen to have a significantly decreased abundance in five of the seven patients studied (Table 3). TGM3 has been noted as being vital for the formation of cornified cell envelope [32] and epidermal terminal differentiation. Expressed in the suprabasal layers of stratified squamous epithelium in skin and mucosa, and regularly expressed in small intestine and brain [33], TGM3 has been implemented in multiple cancer types such as oral squamous cell carcinoma [34], head and neck squamous cell carcinoma [35] and esophageal cancer [36].

Table 3
Refined List of Observed Trends in Saliva Serial Samples Across Varying Time Points, Identified Using label-free LC-MS/MS. Arrows depict an increased abundance (↑) and decrease abundance (↓) in samples from timepoints outlined in Table 2.

Accession number	Protein name	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Q08188	Protein-glutamine gamma-glutamyltransferase E	–	↓	–	↓	↓	↓	↓
P01876	Ig alpha-1 chain C region	↑	↑	↑	↑	↑	↓	↑
Q96DA0	Zymogen granule protein 16 homolog B	↑	↑	–	↑	–	↑	↑
Q9UGM3	Deleted in malignant brain tumors 1 protein	↓	↓	–	–	–	↓	↓
P01023	Alpha-2-macroglobulin	–	↓	–	↓	↓	–	–
P02647	Apolipoprotein A-I	–	↓	–	↓	↓	–	–
P61769	Beta-2-microglobulin	–	–	↓	–	↓	↓	–
P01024	Complement C3	–	–	–	↓	↓	↓	–
Q9UBC9	Small proline-rich protein 3	↓	↑	–	↑	↑	–	↑
P05164	Myeloperoxidase	–	↓	–	↓	↓	–	–
P06702	Protein S100-A9	↓	↓	↑	–	↓	–	↓
P01037	Cystatin-SN	–	–	↑	↑	–	–	–

5. Conclusion

Saliva has been observed to have undetermined potential as a biomarker for disease diagnosis, prognosis and progression. Alongside, saliva also has potential as a source of biomarkers for patient monitoring. MM patients must undergo a bone marrow biopsy, a hugely invasive, uncomfortable process, for diagnosis of disease. Saliva is predicted to show a direct correlation between the protein abundance of both the proteins profile and disease progression from non-malignant to the malignant transformation, especially evident with our identification of β2M in patient saliva samples. We propose that saliva has the potential to predict the need for a bone marrow biopsy procedure to be carried out, as opposed to initially carrying out the invasive procedure, making diagnosis and disease monitoring much less invasive on the patients involved.

Each year, 1% of Monoclonal Gammopathy of Undetermined Significance (MGUS) patients and 10% of Smouldering Multiple Myeloma (SMM) patients transition to symptomatic MM, a malignancy that is considered treatable but rarely curable with current treatment approaches. While clinical, imaging and genomic factors are used to identify an increased risk of progression from MGUS/SMM to MM, utilizing proteomics technologies as a sensitive approach to identify transition associated biomarkers has advantages, potentially providing indicators that signify therapy could begin early and potentially prevent fully developed MM and associated morbidity and mortality. Although further investigation is needed, including larger cohorts of saliva from patients, FABP5 has been identified, in this study, to be a potential saliva biomarker for disease transformation and progression. While there have been advances in MM treatments that have led to improved outcomes, early detection of relapse disease is crucial to allow preventative therapeutic intervention as it could significantly impact on quality of life. Development of biomarkers, either individually or in panels, are crucial in facilitating clinicians to individualize treatment decisions and monitor patients' responses to specific therapies quickly and accurately. Since the malignant B-cells population, along with other cell types in the tumour microenvironment, produce a large amount of proteins that are detectable in circulation, mass spectrometry-based proteomic profiling on biofluid including saliva, a complementary biofluid to blood, displays significant promise to identify biomarkers associated with MGUS, SMM and MM.

Author contribution

CT: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing- Original Draft, Visualisation. DB: Writing-Review & Editing, Project administration, funding acquisition. GL: Resources. PD: Conceptualization, Methodology, Writing- Review & Editing, Supervision. POG: Conceptualization, Resources, Writing-Review & Editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2020.104015.

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