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Proteomic profiling in plasma cell disorders: A feasibility study

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Multiple myeloma (MM) is consistently preceded by precursor states (monoclonal gammopathy of undetermined significance; MGUS, and smoldering multiple myeloma; SMM)¹. Clinically, the risk of progression from MGUS/SMM to MM varies greatly. Indeed, only a subset of patients with MGUS and SMM will eventually progress to MM². While some clinical and laboratory features help in risk stratifying patients with MGUS and SMM, there are no established biomarkers to accurately estimate the risk of progression in these patients³. Available risk stratification models have considerable heterogeneity with significant discordance in predicting risk of progression.

Proteomic profiling could potentially help identify differences in serum protein levels in different stages of the disease and help in risk stratifying patients with plasma cell precursor states. Next generation antibody based techniques can identify and measure a large panel of serum proteins with limited serum samples. Using this approach to measure the levels of a panel of proteins associated with oncogenesis, we investigated the feasibility of characterizing protein profiles in plasma samples from patients MGUS, SMM, and MM.

We identified 17 patients with MGUS, 19 with SMM, and 20 with previously untreated MM. Patients were evaluated at Memorial Sloan Kettering Cancer Center between 12/2010 and 12/2014, had available bio-specimen samples stored in our tissue repository, and provided

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Contribution: O.L., S.M., S.M.D designed the studies; M.P. collected and analyzed the data, N.L, H.H, N.K, H.L., S. M, G.K., D.J.C., O.L, S. G. participated in the clinical care of the patients; and S.M. and O.L. wrote the manuscript; all authors critically read and revised the manuscript.

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informed consent for use of these specimens for clinical research. The study was approved by the Memorial Sloan Kettering Cancer institutional review board.

We used the Proseek Multiplex Oncology II v2, a 96x96 platform (Olink Proteomics) that is based on the Proximity Extension Assay (PEA) technique. PEA is a 96-plex immunoassay that allows high throughput detection of protein biomarkers in liquid samples⁴. 1µl of plasma sample was used from each patient to identify a pre-selected panel of 92 human proteins (full list in the supplementary appendix). For each biomarker, a matched pair of antibodies linked to unique oligonucleotides (proximity probes) binds to the respective protein target. Upon binding, the unique proximity probes hybridize to each other allowing for subsequent detection and quantification by real-time PCR. Linear regression was used to assess the mean increase in biomarker expression across the three patient groups.

Furthermore, false discovery rate (FDR) was used for multiple comparisons adjustment.

Median (range) age for MGUS, SMM, and MM patients was 59 (39–76), 59 (42–70), and 65(44–84) years, respectively (Table 1). The male: female ratio was 12:8, 14:5, and 7:10 respectively (Table 1). Using 1 µl of plasma samples, we used the PEA assay to quantify the levels of 92 proteins in all 56 patients included in this study, thus establishing the feasibility of using this technique for proteomic profiling in plasma cell disorders.

When we conducted univariate statistical testing, 16 proteins (Table 2) were found to have a significant linear trend across the 3 patient groups. This includes proteins previously associated with plasma cell disorders like vascular endothelial growth factor 2 (VEGFR2), vascular endothelial growth factor A (VEGF-A) and interleukin-6. Although the investigation was designed as a feasibility study, we applied a conservative approach in the main model: adjusting for multiple comparisons using FDR, we identified the levels of VEGFR2 to linearly decrease (FDR adjusted p 0.025) from MGUS-->SMM-->MM (Figure 1).

In the absence of available biomarkers that allow risk stratification of patients with MGUS and SMM, we carried out a pilot study to assess the feasibility of measuring a large panel of proteins from limited amounts of plasma samples from patients with plasma cell disorders. Our results show linear trends in the levels of different proteins that have been previously identified as associated with oncogenesis. This pilot study identified VEGFR2 levels linearly decrease from MGUS to MM. A prior study using xenograft tumor models of solid tumors demonstrated an inverse relationship between VEGFR2 levels and tumor size and identified lower VEGFR2 levels as a surrogate marker for tumor growth⁵. Prior research has also identified the frequency of VEGFR2-604TT genotype in advanced stages of MM compared to MM patients with lower disease burden. The role of VEGFR2 polymorphisms, their impact on VEGFR2 levels and risk of plasma cell dyscrasias will need to be better characterized in larger validation studies. VEGF is a growth factor cytokine whose activity is mediated by two receptors, VEGFR1 and VEGFR2. It plays an important role in tumor angiogenesis and previous studies have demonstrated the expression of VEGFR2 in myeloma cell lines and sorted myeloma bone marrow samples⁶. Future larger studies are needed confirm and expand on our results and to confirm these findings. Because of the small sample size and cross-sectional design, this study could not assess correlations

between VEGFR2 expression levels and clinical and genetic characteristics of the patients included. Additional studies that integrate proteomic profiling with existing risk stratification models could more accurately estimate the risk of progression from precursor disease (MGUS/SMM) to MM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Mean expression of VEGFR2 in patients with MGUS, SMM and MM

Table 1

Baseline Characteristics

Characteristics	Multiple myeloma	Smoldering myeloma	MGUS
Age, median (range)	59 (39–76)	57.5 (42–70)	65 (44–84)
Male, n (%)	12 (60)	15 (75%)	7 (41.2)
Heavy chain isotype (IgG/IgM/ IgA/light chain only)	12/0/6/2	14/0/3/3	14/2/1/0
Light chain isotype (K/L)	12/8	11/9	12/5

Table 2

Proteins with a linear trend amongst patients with MM, SMM and MGUS

Biomarker	MM, mean expression (SE)	SMM, mean expression (SE)	MGUS, mean expression (SE)	Unadjusted p-value	FDR adjusted p-value
Vascular endothelial growth factor 2 (VEGFR2)	7.33 (0.04)	7.38 (0.04)	7.57 (0.05)	0.0003	0.025
Furin (Fur)	6.76 (0.08)	6.72 (0.09)	7.18 (0.09)	0.0020	0.071
Hepatocyte growth factor (HGF)	6.64 (0.18)	6.7 (0.1)	7.37 (0.18)	0.0028	0.071
Chorioembryonic antigen (CEA)	2.52 (0.17)	2.14 (0.16)	1.87 (0.11)	0.0033	0.071
Adrenomedullin ⁵	7.2 (0.12)	7.13 (0.09)	7.72 (0.14)	0.0055	0.095
Latency-associated peptide transforming growth factor beta-1 (LAP.TGF β1)	4.59 (0.06)	4.56 (0.13)	5.12 (0.19)	0.0105	0.129
Epithelial cell adhesion molecule (Ep-CAM)	11 (0.25)	10.28 (0.22)	10.18 (0.23)	0.0165	0.129
Cadherin 3 (CDH3)	2.82 (0.09)	2.4 (0.09)	2.48 (0.11)	0.0161	0.129
Fms-related tyrosine kinase 3 ligand (FLT3L)	8.49 (0.1)	8.57 (0.11)	8.92 (0.15)	0.0140	0.129
CD69	6.26 (0.15)	5.98 (0.12)	7.08 (0.34)	0.0178	0.129
Tartrate-resistant acid phosphatase type 5 (TR-AP)	5.06 (0.07)	4.94 (0.08)	5.4 (0.1)	0.0123	0.129
Platelet endothelial cell adhesion molecule (PECAM1)	6.03 (0.06)	6.07 (0.1)	6.4 (0.14)	0.0123	0.129
C-X-C motif chemokine 5 (CXCL5)	4.88 (0.12)	4.98 (0.19)	5.72 (0.39)	0.0229	0.153
Vascular endothelial growth factor A (VEGF-A)	9.4 (0.11)	9.22 (0.1)	9.78 (0.14)	0.0438	0.238
Interleukin-6 (IL-6)	5.34 (0.37)	4.64 (0.21)	6.57 (0.47)	0.0387	0.238
Prolactin (PRL)	4.33 (0.15)	4.57 (0.19)	4.84 (0.18)	0.0435	0.238