Individualized Monitoring of Patients with Monoclonal Gammopathy: Optimizing Sample Preparation for Mass Spectrometry

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Plasma cell proliferative disorders are described by the expansion of a single clone of plasma cells. These cells commonly produce large amounts of monoclonal immunoglobulin. These monoclonal antibodies, clinically referred to as M-protein, are currently detected by agarose gel serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). Patients who test positive for M-protein and exhibit clinical symptoms suggesting multiple myeloma are then subjected to bone marrow biopsy to quantitate the plasma cells. Response to treatments targeting the neoplastic plasma cells from which the M-protein originated are then monitored using the peak area of the M-spike. As the malignant cells are eliminated the M-protein peak will fade into the patient's normal polyclonal background and more sensitive methods including IFE, free light chain assays (FLC) and bone marrow biopsy are used to monitor residual disease. Recently, the Proteomics Core in Mayo Clinic's Department of Laboratory Medicine and Pathology has developed a top down mass spectrometry based method which uses the molecular mass resulting from the patient's unique amino acid sequence to detect and quantify light chain of the M-protein. Using this method the unique mass and increased concentration of the patients M-protein can be distinguished from the polyclonal background providing superior sensitivity in comparison to currently implemented methods; potentially eliminating the need for recurrent bone marrow biopsies. However, before this new methodology can be implemented clinically the sample preparation procedure needs to be refined. Currently, the assay isolates immunoglobulins from serum using Pierce® Melon™ Gel which is designed to enrich IgG but has limitations for other isotypes such as IgA or IgM. To address these limitations, we have optimized and evaluated two different sample preparation procedures, thiophillic absorption

chromatography and bead-based immunoaffinity enrichment. The performance of the three optimized methods was then compared. The results of this comparison indicate that immunoaffinity enrichment is best able to purify all immunoglobulins from serum without bias towards a given isotype. The immunoaffinity enrichment method was then adapted to both MALDI-TOF (matrix assisted laser desorption ionization – time of flight) and ESI-TOF (electro spray ionization – time of flight) mass spectrometry analysis for a comparison of 26 patient samples originally analyzed by SPEP and IFE. 13 of these samples were positive and 13 were negative. All 13 samples positive by SPEP and IFE resulted in positives by MALDI-TOF and ESI-TOF analysis. Interestingly, of the 13 samples that were negative by SPEP and IFE 8 resulted as positive by ESI-TOF and 6 resulted as positive by MALDI-TOF analysis. We have demonstrated that coupling an optimized sample preparation method to the new mass spectrometry based test yields a sensitive and high throughput platform capable of screening for monoclonal gammopathies and monitoring residual disease in myeloma patients.

References

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