Editorial

Monoclonal Gammopathies – Clinical and Laboratory Issues

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The AACB Proteins Workshop held in September 2008 in Adelaide brought together laboratory medical scientists from routine clinical chemistry and immunology laboratories within Australia and New Zealand to discuss the latest laboratory testing in routine protein electrophoresis. The introduction of myeloma therapies such as stem cell transplantation has resulted in more complex electrophoretic patterns and together with the widespread use of the novel therapeutic agents for myeloma has led to increased frequency of protein electrophoresis testing. This issue discusses several clinical and laboratory issues in the diagnosis and monitoring of monoclonal gammopathies.

The monoclonal gammopathies are a group of disorders characterised by the proliferation of clonal plasma cells to produce a monoclonal intact immunoglobulin, free light chain, or free heavy chain protein often referred to as an M-protein, an M-band, or paraprotein. Laboratory quantitation and immunological identification of M-proteins are important in the diagnosis, monitoring and risk stratification of the monoclonal gammopathies which range in severity from the usually benign monoclonal gammopathy of undetermined significance (MGUS) to the incurable multiple myeloma (MM) and light chain amyloidosis (AL amyloidosis). Whereas serum and urine protein electrophoresis (SPEP, UPEP), immunofixation electrophoresis (IFE) and nephelometric or turbidimetric measurement of serum immunoglobulins remain the gold standard laboratory techniques for monitoring of monoclonal gammopathies, new tests such as serum free light chains (FLC) have an increasingly important complementary role, especially where standard tests are inadequate such as in AL and in non-secretory or oligosecretory myeloma.[1]

The paper by Peter Mollee gives an overview of developments in the diagnosis, therapy and monitoring of the monoclonal gammopathies and describes the consensus diagnostic and response recommendations for laboratory testing particularly in multiple myeloma and AL. He discusses the importance of SPEP and UPEP in myeloma monitoring,[2] how in certain clinical situations (e.g. clinical trials and stem cell transplantation) IFE is required to determine accurate reporting of relapse from complete remission, and the importance of measuring serum FLC together with SPEP and UPEP in AL diagnosis and monitoring.[1,3,4]

The usefulness of serum FLC in diagnosis has been demonstrated for several monoclonal light chain diseases and has led the International Myeloma Working Group to recommend SPEP, IFE, and FLC as a sufficient screening panel for plasma cell disorders other than AL.[1] In his paper Jerry Katzmann suggests it is time to change the current screening panels for monoclonal gammopathies. Data from a Mayo Clinic study of 1877 untreated patients with an assortment of plasma cell disorders are presented that compare the diagnostic sensitivity of a number of monoclonal gammopathy screening panels. Single tests detected between 74.3% and 87.0% of M-proteins and the complete panel of SPEP, IFE, and FLC combined with urine IFE and UPEP detected 98.6%. SPEP and FLC together identified 94.3% of M-proteins; addition of IFE increased this proportion to 97.4%, but the majority of those missed M-proteins were MGUS at low risk of progression to MM. Hence, because only a small increase in sensitivity is provided by urine studies and serum IFE, the use of SPEP plus FLC provides a simple and efficient initial diagnostic screen for the high tumour burden monoclonal gammopathies. The limitation of such a study is that the screening population was limited to patients with documented monoclonal gammopathies rather than the general population in whom a monoclonal gammopathy may be suspected. As such, the Mayo study focuses on screening panel sensitivity but not specificity. Whether such proposed screening panels will prove useful in terms of sensitivity and specificity in the general population will need to be assessed by future studies.

The paper by Hall et al. entitled 'Significance of abnormal protein bands in patients with multiple myeloma following autologous stem cell transplantation', shows the complexity of serum protein electrophoresis patterns in patients being monitored for M-protein post-stem cell transplantation. Of 49 myeloma cases, 73% developed small abnormal protein bands (APB) post-transplantation which included 54 episodes of oligoclonal bands and 15 episodes of apparent monoclonal bands, but with progression to frank disease in only one case. It is suggested that the initial development of APB is associated with marked reduction in the malignant plasma cell clone as evidenced by the achievement of complete remission and that it may be a surrogate marker for myeloma eradication. In assessing APB, isoelectric focusing proved a useful tool in that it immediately demonstrated that 54 of 69 APB were oligoclonal and thus were not clinically concerning for relapsed disease.

Turning to the laboratory measurements of serum and urine M-proteins, Margaret Jenkins describes the evolution of serum electrophoresis from the Tiselius moving boundary liquid system to the semi-automated high resolution agarose gel methods through to fully automated capillary electrophoresis. Examples of M-protein detection using contemporary techniques are given in conjunction with their identification by IFE and isoelectric focusing (IEF) methods. Findley Cornell then goes on to describe in detail the exquisite resolution and high sensitivity of IEF, its ability to distinguish between monoclonal, oligoclonal and polyclonal patterns, and its much higher sensitivity compared with zone electrophoresis in detecting low levels of mono and oligoclonal immunoglobulin when superimposed on a normal polyclonal background. When combined with blotting and probing techniques IEF can detect down to μg/L protein levels. While automated routine protein electrophoresis techniques are satisfactory for detecting and identifying most M-proteins, IEF is a useful ancillary method for interpreting more complex patterns.

Tate et al. describe the analytical issues experienced with the commercial quantitative serum FLC assay. Factors such as assay imprecision, lot-to-lot variation of FLC reagent, method differences and underestimation of FLC due to nonlinearity of the monoclonal protein or to antigen excess may have a significant effect when monitoring therapy. For example, nonlinear FLC may require several sample dilutions often 4- to 80-fold higher than the initial analyser dilution to reach a final FLC concentration. Laboratory staff and clinicians should be aware of the analytical limitations of the FLC assay as falsely low values may mislead clinical diagnosis and assessment of response to therapy.

Apart from the analytical aspects of M-protein measurement, appropriate post-analytical interpretation of results and individualised patient commenting is an important part of protein electrophoresis reporting. As indicated already in several of the papers, laboratories should take particular care with the choice of terms for reporting very small bands (e.g. $\leq$2 g/L), particularly in the post-autologous stem cell transplant setting. Reports require some comment as to the significance of the result to avoid a small abnormal band labelled as "monoclonal" being wrongly interpreted to mean relapse.

In the paper entitled 'Reporting of quantitative protein electrophoreis in Australia and New Zealand: a call for standardisation', Inman et al. describe the results of two questionnaire surveys on protein electrophoresis reporting practices by 30 routine clinical laboratories. The report shows that there exists: 1) considerable variation in the quantification of urine Bence Jones protein and of M-proteins comigrating with other serum proteins; 2) inconsistent commenting on the presence of M-proteins; and 3) a lack of adherence to the clinical response criteria guidelines for confirmatory laboratory testing. Although, as the authors note, there were some limitations of the surveys, they make the important point of the need for a standardised approach to the reporting of protein electrophoresis. Standardised reporting practices would benefit both the clinician and the patient, and enable the use of uniform codes for electronic laboratory information transfer.

In conclusion, the papers presented in this Proteins issue highlight the importance of a complementary clinical and laboratory approach to the diagnosis and monitoring of monoclonal gammopathies. The laboratory has a major role to play both in analysis of M-proteins and providing informative reporting of protein electrophoresis, while ensuring a close collaboration with local clinicians.

References

1. Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia 2009;23:215-24.
2. Durie BG, Harousseau J-L, Miguel JS, Bladé J, Barlogie B, Anderson K, et al. International uniform response criteria for multiple myeloma. Leukemia 2006;20: 1467-73.
3. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion

from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol 2005;79:319-28.

4. Palladini G, Russo P, Bosoni T, Verga L, Sarais G, Lavatelli F, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. Clin Chem 2009;55:499-504.