Laboratory Evaluation of Plasma Cell Neoplasms
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In this issue we review the laboratory work up of patients suspected of having a plasma cell neoplasm. As the range in clinical behavior is quite broad among plasma cell neoplasms, few generalizations apply. Besides timely and accurate screening and diagnosis, the laboratory must provide data relevant to prognosis, staging, risk stratification, and post-therapy monitoring. The following reflects consensus criteria defined by the International Myeloma Working Group (IMWG) (Blood 2011 vol. 117 no. 18: 4701-4705; Leukemia 2009 vol. 23: 3-9), the Mayo Clinic (www.msmart.org), and the 2008 WHO classification of tumors of hematopoietic and lymphoid tissues (WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues, IARC Press; 2008:200–213). Also, two excellent reviews by Robert Kyle and S. Vincent Rajkumar are quoted liberally (BJ Haem 2007 vol 139 (5): 730-743, and Leukemia 2009 vol. 23: 3-9).

Classification of Plasma cell Disorders (Monoclonal Gammopathies)

Plasma cell neoplasms comprise a spectrum of monoclonal plasma cell proliferative disorders ranging in clinical severity from benign to highly malignant. Sub-classification is based on clinical and laboratory criteria:

Three Major Groups of Monoclonal Gammopathies:

Monoclonal Gammopathy of Undetermined Significance (MGUS): A common (3% over 50, 5% over 70, 8% over 85 years) premalignant plasma cell disorder associated with a constant lifelong risk of multiple myeloma of 1% per year. Definition: serum monoclonal protein <3 g/dL and clonal plasma cells <10% in the bone marrow and absence of CRAB*. The risk of progression to multiple myeloma (MM) is highly dependent on (1) an abnormal serum free light chain (FLC) assay, (2) the presence of a non-IgG paraprotein, and (3) an M protein >1.5 g/dL.

Risk of progression to MM at 20 years: 3 risk factors: 58%; 2 risk factors: 37%; 1 risk factor: 21%; no risk factors: 5%.

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Information for Providers

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Smoldering Multiple Myeloma (SMM): Asymptomatic plasma cell disorder with serum monoclonal protein (almost always IgG or IgA) >3 g/dL and/or clonal plasma cells >10% in the bone marrow and absence of CRAB.* SMM is essentially asymptomatic multiple myeloma.

Risk of progression to MM: 51% at 5 years, 66% at 10 years, 73% at 15 years.

Multiple Myeloma (MM): A plasma cell malignancy accounting for up to 15% of all US hematologic malignancies. In almost all cases MM probably evolves from pre-existing MGUS. MM is genetic, phenotypically, and clinically heterogeneous, with median overall survivals ranging from 6 months to over 10 years. Definition: serum and/or urinary monoclonal protein; usually >10% clonal plasma cells in the bone marrow and symptoms of tissue impairment (CRAB*). In the 2008 WHO classification the minimal percentage of plasma cells required for a diagnosis of plasma cell myeloma was lowered from 30% to 10%. In fact, most authorities no longer require a minimal percentage of abnormal plasma cells for a diagnosis of MM in patients who are symptomatic.

*CRAB = hyperCalcemia, Renal insufficiency, Anemia, or Bone lesions.

Additional plasma cell disorders:
Other less common plasma cell disorders include solitary plasmacytoma, primary AL (light chain) amyloidosis, and the POEMS syndrome (Polyneuropathy, Osteosclerosis, Endocrinopathy, M-protein). The latter disorders are distinguished from MM, SMM, and MGUS by specific clinical and radiologic findings.

Monoclonal Gammopathy – To Screen or Not to Screen
As noted, MGUS is increasingly prevalent with age, yet the risk of evolution to multiple myeloma (MM) is quite low. Therefore, screening of healthy individuals as part of routine medical care is discouraged, as the finding of a monoclonal gammopathy may lead to significant expense and patient anxiety for a condition that in most instances will not require treatment and will have no impact on the patient’s life expectancy. Screening is appropriate for patients undergoing evaluation for a disorder in which a plasma cell neoplasm is in the differential diagnosis (particularly those with symptoms of CRAB*). For screening purposes, the combination of serum protein electrophoresis, immunofixation, and serum free light chain analysis is sufficiently sensitive to detect nearly all clinically significant monoclonal gammopathies, including oligosecretory myeloma and light chain-only myeloma (possible exception: AL amyloidosis). If a monoclonal protein is detected and MM is a possibility, testing can be expanded to include:

Laboratory Tests Useful in Evaluating Suspected Multiple Myeloma

CBC with differential and peripheral blood smear morphology. Significant rouleaux in the blood smear of a patient with normocytic anemia often reflects an underlying monoclonal gammopathy, particularly if the pathologist detects circulating atypical plasma cells. In some cases flow cytometry can detect clonal plasma cells in the blood of patients with MM, although for most patients flow analysis is better reserved for bone marrow evaluation. There is no value in confirming rouleaux by assessment of the erythrocyte sedimentation (ESR) rate.
**Chemistries:** Calcium, creatinine, electrolytes, liver function tests, albumin, beta-2-microglobulin, LDH are appropriate. The three latter tests have staging and prognostic value. The International Staging System (ISS) based only on the serum albumin and beta-2-microglobulin levels has replaced the classical Durie-Salmon system in patients with MM:

ISS stage I: beta-2-microglobulin <3.5 mg/L plus serum albumin ≥ 3.5 g/dL (median survival, 62 months); stage II: neither stage I nor III (median survival, 44 months); and stage III: beta-2-microglobulin ≥ 5.5 mg/L (median survival, 29 months).

**Serum protein electrophoresis (SPEP)** with densitometric quantification and immunofixation (IFX) are essential to establish the presence, quantity, and type of monoclonal protein. IFX is the gold standard for the qualitative demonstration of a serum monoclonal protein. The heavy chain type as determined by IFX has prognostic (IgA worse than IgG) and diagnostic significance. An IgM paraprotein almost always suggests a low-grade B-cell lymphoproliferative disorder such as Waldenstrom's macroglobulinemia (if the M protein is large), or splenic marginal zone lymphoma or CLL (if the M protein is small), while an IgG or IgA suggests a plasma cell disorder. The presence of an IgD monoclonal protein almost always indicates MM, AL, or plasma cell leukemia. Finally, the quantity of monoclonal protein is needed for classification (MGUS vs. SMM vs. MM) and is prognostic, as the level of paraprotein reflects tumor burden.

**Quantitation of serum immunoglobulins** by nephelometry complements protein electrophoresis, has prognostic significance, and may demonstrate reductions in normal immunoglobulins.

**Serum free light chain (FLC) analysis**
Since 2001 FLC analysis has become increasingly important in the diagnosis, monitoring, and prognosis of patients with monoclonal plasma cell disorders. Clonal light chains must exceed a concentration of at least 500 mg/L to be detected by SPEP, or 150 mg/L to be detected by serum IFX. In contrast, FLC assays detect light chain concentrations as low as 3 mg/L, and therefore may be positive in light-chain-related disorders despite negative results on SPEP or IFX. High baseline levels of clonal FLCs are associated with poor prognosis in all plasma cell disorders. In patients with MGUS and solitary plasmacytoma an abnormal FLC result carries an increased risk of progression to MM.

**Cost-effectiveness of free light chain analysis**
Serum FLC assays are more cost-effective than urine tests in screening for monoclonal gammopathy (medicare reimbursement is $38 for the serum FLC assay vs $71 for a complete urine assay, including total urine protein, urine protein electrophoresis, and urine IFX).

**Use of Free Light Chain Analysis**
Current assays measure free kappa (0.33-1.94 mg/dL) and lambda (0.57-2.63 mg/dL) separately and express these as a ratio (0.26-1.65). An adjusted ratio is used for patients with renal impairment (0.37-3.1). A FLC ratio <0.26 indicates the presence of a clonal lambda free light chain, while a ratio >1.65 indicates a clonal kappa protein. FLC is essential in patients with nonsecretory or oligosecretory MM, as well as in light chain-only MM. FLC analysis has three main uses: (1) it is prognostic in MM, MGUS, and SMM; (2) it can replace the standard 24-hour urine protein study in the initial screening phase in a patient suspected of MM; (3) it
may be a sufficiently sensitive marker of disease burden to replace serial bone marrow examinations for the monitoring of MM post-therapy (particularly patients with oligosecretory MM).

**Urinalysis**

Routine urinalysis is essential in the initial evaluation of a patient suspected of having MM. IMWG guidelines stipulate the combination of serum FLC analysis, SPEP, and IFX can replace the standard 24-hour urine protein study in the diagnostic work up of plasma cell disorders (all except AL amyloidosis). If a clonal plasma cell disorder is subsequently diagnosed, however, a 24-hour urine collection with urine total protein (UTP), electrophoresis (UPEP), and IFX are needed.

**Bone Marrow Evaluation in Patients with a Documented Monoclonal Gammopathy**

Once a monoclonal gammopathy has been documented, physicians often request bone marrow examination. Bone marrow examination is indicated in any patient with a monoclonal gammopathy who has CRAB symptoms, and in those whose paraprotein exceeds 1.5 g/dL, have an abnormal FLC study, or have a non-IgG (IgA, IgD, or IgM) paraprotein. However, in MGUS patients with an IgG paraprotein <1.5 g/dL, and who are free of CRAB symptoms, bone marrow examination is rarely indicated. Finally, FLC analysis may be sensitive enough to suffice for serial monitoring of patients post-therapy in lieu of repeated bone marrow examinations.

Bone marrow aspiration and biopsy, with immunophenotypic analysis, conventional cytogenetics and FISH should be obtained on all newly-diagnosed MM patients and in those with monoclonal gammopathy, except as noted above. The % plasma cells cannot be obtained by flow cytometry but should be based on the highest of two methods:

1. 500 cell manual differential using the aspirate preparation that yields the highest % plasma cells.
2. Trephine biopsy stained by immunohistochemistry (IHC) for CD138 with % estimate of plasma cell infiltrate.

When both procedures are performed, the highest % of plasma cells by either is used for diagnosis.

**Flow Cytometry**

Patients with newly-diagnosed should have a complete immunophenotypic analysis performed on bone marrow aspirate. Physicians may request flow cytometry specifically when ordering a bone marrow or may order it “per pathologist,” deferring to the morphologic findings. The Alina Flow Cytometry facility utilizes multiparameter panels for the complete characterization of plasma cell disorders. Flow panels are designed to distinguish neoplastic from reactive plasmacytosis, establish light chain restriction, define heavy chain isotype, identify aberrant phenotypic patterns useful in post-therapy monitoring, and exclude other potential B-cell malignancies that may present as a monoclonal gammopathy.

There is increasing awareness that monoclonal plasma cell disorders are genetically and im-
munophenotypically heterogeneous, and that this heterogeneity can be exploited for disease-monitoring purposes. The detection of residual disease by multiparameter flow cytometry is emerging as a powerful prognostic marker in MM patients following autologous stem cell transplantation (Rawstron, et. al., Haematologica 2008, vol 93 (3): 431-438).

Genetics

Conventional (metaphase) cytogenetics are abnormal in <20% of bone marrow aspirates in patients with MM, with even lower rates in MGUS/SMM, although we now know from research using interphase fluorescence in situ hybridization (FISH) and DNA flow cytometry (ploidy analysis) that complex chromosomal abnormalities are universal in MM. The use of CD138 immunomagnetic beads to purify plasma cells from bone marrow aspirates has extended the reach of FISH analysis in bone marrow samples to include patients with MGUS and SM, as well as MM. Although the sensitivity of cytogenetics is low in all plasma cell disorders, it remains indicated in the evaluation of patients suspected of having MM, as the finding of a clonal abnormality carries significant negative prognostic importance. In addition, cytogenetic studies can separate hyperdiploid from nonhyperdiploid patients (see below) and detects uncommon additions, deletions, and translocations.

Research has revealed two prognostically relevant pathways in plasma cell oncogenesis:

Hyperdiploid Multiple Myeloma
48 – 74 chromosomes reflecting multiple recurrent trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19.

Nonhyperdiploid Multiple Myeloma
Hypo- and pseudo-diploid, and near-tetraploid: <48 or >74 chromosomes, frequently having translocations involving 14q32 and abnormalities of chromosome 13 (-13/13q-).

Prognostic Impact of Genetic Findings in Multiple Myeloma

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<thead>
<tr>
<th>Parameter</th>
<th>Effect</th>
<th>Risk Group</th>
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<tr>
<td>Hyperdiploidy</td>
<td>48 – 74 chromo</td>
<td>Standard</td>
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<tr>
<td>Nonhyperdiploidy</td>
<td>&lt;48 or &gt;74 chromo</td>
<td>Intermediate</td>
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<tr>
<td>Abnormal 13</td>
<td>-13/13q-</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Deletion 17p</td>
<td>p53 gene deletion</td>
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<tr>
<td>GEP*</td>
<td>High risk signature</td>
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**Fluorescence in situ hybridization (FISH) Findings**

<table>
<thead>
<tr>
<th>IgH translocations</th>
<th>Dysregulated Gene</th>
<th>Risk Group</th>
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<tr>
<td>t(11;14)(q13;q32)</td>
<td>CCND1 (cyclin D1)</td>
<td>Standard</td>
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<td>t(6;14)(p21;q32)</td>
<td>CCND3 (cyclin D3)</td>
<td>Standard</td>
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<td>t(4;14)(p16;q32)</td>
<td>FGFR-3 and MMSET</td>
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<td>t(14;16)(q32;q23)</td>
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<tr>
<td>t(14;20)(q32;q11)</td>
<td>MAFB</td>
<td>High</td>
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**Other FISH: Finding:**
- Deletion 17p
- P53 gene deletion

*GEP = Gene expression profiling. Highly prognostic in MM but not widely available.

All above (except GEP) are available through the Allina Medical Laboratories Cytogenetics Laboratory. Both FISH and conventional cytogenetics are recommended in the diagnostic work up of patients suspected of having MM. Besides objectively confirming the diagnosis of a clonal plasma cell neoplasm, the findings are prognostic in patients undergoing therapy for MM.

**Genetics and Disease Progression**

Patients with MGUS/SMM share similar abnormalities by FISH and cytogenetics to those with MM, suggesting the genetic lesions observed in these disorders are common initiating abnormalities among plasma cell neoplasms generally, but are not related to disease progression. Therefore, FISH is not useful in sub-classifying plasma cell neoplasms or in estimating prognosis in patients other than in those with MM. Nevertheless, FISH based on CD138-purified plasma cell fractions is extremely sensitive in detecting a clonal plasma cell population, and may have utility as a diagnostic aid in bone marrow samples when flow cytometry, light microscopy, and immunohistochemistry are inconclusive.

**Other Potentially Useful Tests in Patients with MM**

**Plasma cell Labeling Index (LI):** An elevated LI is a strong adverse prognostic marker in patients with MM and may be helpful in the treatment planning of selected patients. This parameter is available from Mayo Labs but requires a special transport kit and therefore must be requested prospectively before bone marrow examination.

**Disease Monitoring**

Recent studies suggest combined serum (SPE, IFX, FLC) and urine (UPEP) studies are adequate for disease monitoring of most MM patients post-therapy. Bone marrow examination can be reserved for patients clinically suspected of having residual or recurrent disease, for patients with nonsecretory MM, and for those in whom detection of residual disease would lead to a change in therapy.

*For questions, comments, or suggestions about this newsletter or other laboratory issues, please contact Lauren Anthony, MD, Medical Director of Allina Medical Laboratories, (612) 262-5013 or Lauren.Anthony@allina.com*