

GUIDELINES FOR STANDARD INVESTIGATIVE WORKUP: Report of the International Myeloma Workshop Consensus Panel 3

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Minimal diagnostic and prognostic tests

Initial investigation of a patient with suspected multiple myeloma should include the tests shown in Table 1. Family history should focus on first degree relatives with the diagnosis of hematologic malignancies especially lymphoma, chronic lymphocytic leukemia and plasma cell dyscrasias. Past medical history should focus on comorbid conditions that may affect treatment decisions such as coronary artery disease, congestive heart failure, deep venous thrombosis, hypertension, renal disorders, liver disorders, lung diseases etc. A complete blood count with differential should be ordered and a peripheral blood smear should be evaluated in search of specific findings such as rouleaux formation, and circulating plasma cells. A complete biochemistry screen should be ordered which include liver function tests, renal function tests, electrolytes, calcium and albumin.

Both serum and urine should be assessed for monoclonal protein. Agarose gel electrophoresis or capillary zone electrophoresis of serum and urine is preferred to screen for the presence of monoclonal protein. Importantly, quantitation of serum immunoglobulins by nephelometry should also be performed. Measurement of monoclonal protein both by densitometer tracing and by nephelometric quantitation is recommended. These two tests are complementary and nephelometric quantitation may be particularly useful for low levels of uninvolved immunoglobulins. However, it should be noted that nephelometric quantitation may overestimate the monoclonal protein concentration when its value is high (Riches et al). Serum immunofixation is the gold standard method to confirm the presence of a monoclonal protein and to distinguish its heavy and light chain type. Serum immunofixation should also be performed when there is hypogammaglobulinemia (a frequent finding in light chain only myeloma) or even when the serum electrophoretic

pattern appears normal if there is a suspicion of multiple myeloma or a related disorder. Low levels of monoclonal proteins may be associated with a normal serum electrophoresis. When a patient has only monoclonal light chain or has a monoclonal serum protein but the immunofixation is negative for IgG, IgA or IgM, the possibility of IgD or IgE monoclonal immunoglobulin must be considered. If only a monoclonal light chain is found, immunofixation for IgD and IgE may be performed and if positive for IgD or IgE, then quantitation of these immunoglobulins follows. This approach eliminates the need for quantitation of IgD or IgE in many instances. Immunosubtraction has been used in place of Immunofixation electrophoresis but is less sensitive and is being supplanted by IFE.

The quantitation of serum albumin is important since albumin is a key component of the currently used International Staging System for multiple myeloma. The most accurate method to measure serum albumin is by nephelometry but this approach is not widely used. Serum albumin can be measured by densitometry from the electrophoretic strip. However its value can be affected by the level of the monoclonal protein: high concentrations of monoclonal protein tend to overestimate the concentration of serum albumin (Snozek). Serum albumin can also be measured with bromcresol, which is the method used in some laboratories when serum albumin is ordered in a chemistry panel. This assays show good correlation with the gold standard nephelometric quantitation, and is independent of the monoclonal protein levels. A recent study indicated that all albumin methods perform similarly in predicting survival and may be used in prognostication by the ISS (Kapoor).

Routine urinalysis is important in suspected myeloma. For screening a random urine protein electrophoresis and urine immunofixation may be performed. Once a diagnosis of myeloma is suspected or established, all patients should undergo 24 hour urine collection to calculate the amount of proteinuria. An aliquot from an adequately concentrated 24 hour specimen should be sent for electrophoresis. A monoclonal protein appears as a homogeneous peak in the densitometer tracing. Its concentration can be calculated on the basis of the size of the peak and the amount of total protein in the 24 hour urine specimen. Immunofixation of an aliquot from a

concentrated 24 hour urine collection is required to confirm the presence and type of heavy and light chain. Immunofixation should be performed even if there is no measurable protein and even if there is no peak on urine electrophoresis. A 24 hour urine collection cannot be replaced by a morning urine sample. The use of random urine samples with analytes corrected relative to creatinine concentration requires further evaluation but cannot be recommended at this point. Measurement of urine free light chain levels or urine total kappa and total lambda levels is not recommended.

Serum free light chain assay, as it becomes widely available, is recommended in all newly diagnosed patients with plasma cell dyscrasias. Measurement of serum free light chain is very important in patients with non-secretory multiple myeloma ie with negative serum and urine immunofixation and in patients who secrete small amounts of monoclonal protein in the serum and/or urine (oligosecretory myeloma), as well as in light chain only myeloma. Serum free light chain estimation does not obviate the need for 24 hour urine studies. Serum free light chains may be useful in patients with solitary plasmacytoma (Dingli et al, 2006) or with smoldering (asymptomatic) myeloma (Dispenzieri et al, 2008.) because an abnormal value may be associated with a higher risk of progression to symptomatic myeloma. Testing for serum free light chains is also recommended for patients with monoclonal gammopathy of undetermined significance as it helps predict probability of progression to multiple myeloma. Urine free light chain assay should not be performed.

A patient with suspected multiple myeloma should undergo a unilateral bone marrow aspirate and/or biopsy and the diagnosis is confirmed when over 10% clonal plasma cells are detected. Clonality of plasma cells should be established by identification of a monoclonal immunoglobulin in the cytoplasm of plasma cells by immunoperoxidase staining or by immunofluorescence. Immunophenotyping by flow cytometry is performed by some centers but this technique may not be widely available and standardized for general use. While bone marrow aspirate alone may be sufficient to confirm the diagnosis, a trephine biopsy should be considered during the same procedure for the following reasons a) it may provide a more reliable assessment of plasma cell infiltration (Rajkumar) b) it may obviate the need

for a repeat procedure should the bone marrow aspirate prove to be inadequate. When both procedures are performed the highest number of plasma cells obtained by either procedure is recorded for the purpose of diagnosis.

Standard metaphase cytogenetics should be included in the initial assessment of a patient with high suspicion of multiple myeloma. Despite the low yield of this method (20% or less), it can provide useful prognostic information by separating hyperdiploid from non-hyperdiploid patients and can capture uncommon additions, deletions and translocations. Furthermore patients should undergo fluorescent in situ hybridization (FISH) preferably after sorting of plasma cells with probes that includes chromosome 17p13, t(4;14), and t(14;16).

While some tests are not required for the diagnosis of myeloma, they are important for prognosis or staging. As such the following tests are recommended: serum b-2 microglobulin which reflects tumor burden and forms the basis for the International Staging System and serum LDH which has an independent prognostic significance in several studies. Assessment of erythrocyte sedimentation rate does not provide additional information and is not required. Although C-reactive protein is not useful for the risk assessment of myeloma, it may be helpful when an infection is suspected.

The skeletal survey remains the standard method for imaging screening at diagnosis, is readily available at modest cost, allows large areas of the skeleton to be assessed and may detect long bone lesions at risk of impending fracture. Plain radiographs should include a postero-anterior view of the chest, antero-posterior and lateral views of the cervical, thoracic and lumbar spine, humeri and femora, anteroposterior and lateral view of the skull and anteroposterior view of the pelvis.

Magnetic resonance imaging is a non-invasive technique which provides detailed information about bone marrow involvement and its pattern (focal, diffuse, variegated), is useful for the assessment of the extent and nature of soft tissue disease arising from bone lesions, and can detect unsuspected, asymptomatic lesions. An MRI of the spine and pelvis is mandatory in all patients with a presumed diagnosis of solitary plasmacytoma. An MRI is also recommended in patients with smoldering (asymptomatic)

myeloma because it can detect occult lesions and, if positive, can predict for more rapid progression to symptomatic myeloma. MRI can be considered in patients with symptomatic myeloma as routine evaluation because: a) Unsuspected focal lesion and soft tissue plasmacytomas involving the spine and pelvis can be visualized; and b) patterns of MRI abnormality (ie diffuse pattern or a high number of focal lesions) may have prognostic significance. However, MRI is mandatory in symptomatic patients for a detailed evaluation of a painful area of the skeleton to look for a soft tissue mass arising from a bone lesion or for the investigation of patients with a suspicion of cord compression, providing an accurate assessment of the level and extent of cord or nerve root compression, size of the tumor mass and degree to which it may affect the epidural space. An MRI of the spine is valuable in defining the etiology of new, painful collapsed vertebra ie due to osteoporosis or due to myelomatous involvement. Osteoporosis with compression fracture requires thorough evaluation with an MRI. If a focal myelomatous lesion is detected, then the patient has symptomatic myeloma which requires treatment. However, if the fracture is due to osteoporosis (especially in certain populations such as elderly white women), then other criteria such as degree of marrow infiltration, anemia etc. should be considered in order to diagnose symptomatic myeloma. Occasionally, an CT-guided biopsy of the collapsed vertebra is needed to make the diagnosis. Furthermore, an MRI is strongly indicated in patients with non-secretory myeloma for their initial assessment, follow-up of response to treatment as well as relapse.

The role of PET-CT is yet to be clearly defined in multiple myeloma. It is helpful for detection of extraosseous soft tissue masses and evaluation of rib and appendicular bone lesions. PET-CT is especially useful in patients with elevated LDH, Bence Jones protein escape and otherwise rapidly recurrent disease or with suspected extramedullary plasmacytoma. Unlike MRI, PET-CT obviates the need for a skeletal survey. There is recent evidence that the combination of PET-CT and MRI may improve the diagnostic accuracy of solitary plasmacytoma but is not yet recommended (Salaun et al).

Finally, specific tests may be required during the initial assessment of a patient with a suspected myeloma. When the degree of anemia is out of proportion of the myeloma tumor load, other co-existent causes need to be

looked for such as iron deficiency, vitamin deficiency etc. When mild or moderate hypercalcemia is detected and no typical myeloma bone lesions are seen the possibility of primary hyperparathyroidism should be ruled out with measurement of serum PTH. Finally when there is non-selective proteinuria, unexplained weight loss, low ECG voltages and left ventricular hypertrophy on echocardiogram, congestive heart failure, unexplained hepatomegaly, elevated alkaline phosphatase and G-GT, symptoms and signs of peripheral or autonomic neuropathy or carpal tunnel syndrome, the possibility of primary systemic amyloidosis (AL) should be considered by specific staining of subcutaneous fat aspirate and bone marrow. Biopsy of a suspected organ may be necessary in some instances. In some myeloma patients with diabetes or hypertension who present with non-selective proteinuria associated with mild to moderate but stable renal impairment a renal biopsy may be indicated to determine etiology of the renal lesion and its possible association to a plasma cell disorder. Furthermore, non-selective proteinuria without evidence of amyloidosis in a patient with plasma cell dyscrasia may be secondary to immunoglobulin deposition disease. In such a case, a renal biopsy with appropriate studies is necessary. Routine testing for hyperviscosity is not recommended. The plasma hyperviscosity as determined by testing does not correlate with clinical manifestations of hyperviscosity. Funduscopic examination is more helpful in defining clinically significant hyperviscosity. Hyperviscosity in IgG myeloma is rare unless it is IgG subclass 3. Simple numerical values of test results for hyperviscosity do not warrant clinical intervention with plasmapheresis etc.

Follow-up investigation after therapy

For patients with measurable monoclonal protein in serum both electrophoretic studies and quantitative immunoglobulins are recommended to assess response although electrophoretic measurements to follow monoclonal protein is preferred. For several patients, especially with IgA, IgM or IgD myeloma, nephelometric quantitation of serum immunoglobulin is necessary. It is however important for a particular patient to use the same method for the follow-up of his disease. For patients with light chain myeloma,

24 hour urine collection with total protein and urine electrophoresis to quantify Bence Jones proteinuria is recommended. For patients with non-secretory or oligosecretory myeloma, the free light chains should be serially assessed. When documentation of response or of relapse is in doubt, evaluation with MRI may be helpful. For most patients there is no necessity for bone marrow examination to assess response provided that the myeloma can be monitored with serum and urine studies and there is no indication to change the patient's treatment. Bone marrow aspiration and/or biopsy is indicated to establish complete response. Complete response has prognostic implication since several studies have indicated that it may predict for longer duration of response and survival. Furthermore there is no indication to repeat the metaphase karyotype, FISH studies or flow cytometric studies as a routine follow-up. There is no need to repeat the skeletal survey in a patient who is responding to treatment unless he develops bone symptoms.

Test to be performed at relapse

Most of the workup recommended at diagnosis is also pertinent at relapse. The prognostic significance of b2 microglobulin or ISS at relapse is not clear. Elevated serum LDH is predictive of poor prognosis. A bone marrow aspirate and/or biopsy should be performed if clinically indicated ie suspicion of hyposecretory myeloma progression or when a myelodysplastic syndrome is considered (presence of cytopenias). For patients who did not have cytogenetic or FISH analyses at baseline, these tests should be performed at relapse. Furthermore, if cytogenetic and/or FISH analyses were performed at baseline and were normal, these tests should be performed at relapse. However, if a patient already had an identified high risk feature on cytogenetic or FISH analyses, then there may be no need to look for it again at relapse. There is evidence that some novel agent-based treatments may be more effective than others in patients with adverse cytogenetic features. A skeletal survey may be indicated to detect possible lesions at risk for fracture. Other imaging studies (CT, MRI, PET/CT) to detect soft tissue masses arising from

bone lesions or extramedullary disease may be indicated according to clinical circumstances.

REFERENCES

Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Haematol.* 2003;121:749-757.

Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. *N Engl J Med.* 2007;356:2582-2590.

Smith A, Wisloff F, Samson D. Guidelines on the diagnosis and management of multiple myeloma 2005. *Br J Haematol.* 2006;132:410-451.

Rajkumar SV, Fonseca R, Dispenzieri A, et al. Methods for estimation of bone marrow plasma cell involvement in myeloma: predictive value for response and survival in patients undergoing autologous stem cell transplantation. *Am J Hematol.* 2001;68:269-275.

Fonti R, Salvatore B, Quarantelli M, et al. 18F-FDG PET/CT, 99mTc-MIBI, and MRI in evaluation of patients with multiple myeloma. *J Nucl Med.* 2008;49:195-200.

Zamagni E, Nanni C, Patriarca F, et al. A prospective comparison of 18F-fluorodeoxyglucose positron emission tomography-computed tomography, magnetic resonance imaging and whole-body planar radiographs in the

assessment of bone disease in newly diagnosed multiple myeloma. *Haematologica*. 2007;92:50-55.

Wiesenthal AA, Nguyen BD. F-18 FDG PET/CT staging of multiple myeloma with diffuse osseous and extramedullary lesions. *Clin Nucl Med*. 2007;32:797-801.

Walker R, Barlogie B, Haessler J, et al. Magnetic resonance imaging in multiple myeloma: diagnostic and clinical implications. *J Clin Oncol*. 2007;25:1121-1128.

Moulopoulos LA, Gika D, Anagnostopoulos A, et al. Prognostic significance of magnetic resonance imaging of bone marrow in previously untreated patients with multiple myeloma. *Ann Oncol*. 2005;16:1824-1828.

Bredella MA, Steinbach L, Caputo G, Segall G, Hawkins R. Value of FDG PET in the assessment of patients with multiple myeloma. *AJR Am J Roentgenol*. 2005;184:1199-1204.

Baur-Melnyk A, Buhmann S, Durr HR, Reiser M. Role of MRI for the diagnosis and prognosis of multiple myeloma. *Eur J Radiol*. 2005;55:56-63.

Mariette X, Zagdanski AM, Guermazi A, et al. Prognostic value of vertebral lesions detected by magnetic resonance imaging in patients with stage I multiple myeloma. *Br J Haematol*. 1999;104:723-729.

Moulopoulos LA, Dimopoulos MA, Smith TL, et al. Prognostic significance of magnetic resonance imaging in patients with asymptomatic multiple myeloma. *J Clin Oncol*. 1995;13:251-256.

Drayson M, Tang LX, Drew R, et al. Serum free light chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. *Blood* 2001;97:2900-2902

Kyle RA, Rajkumar SV. Multiple myeloma. *N. Engl J Med* 2004;351:1860-1873

Kyle RA. Sequence of testing for monoclonal gammopathies. Serum and urine assays. *Archives of Pathology and Laboratory Medicine* 1999;123:114-118

Katzmann JA, Clarck RJ, Abraham RS, et al. Serum reference and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chain. *Clinical Chemistry* 2002;48:1437-1444

Moulopoulos LA, Dimopoulos MA, Weber D, et al. Magnetic resonance imaging in the staging of solitary plasmacytoma of bone. *J Clin Oncol* 1993;11:1311-1315

Snozek CL, Saenger AK, Greipp PR, et al. Comparison of bromocresol green and agarose protein electrophoresis for quantitation of serum albumin in multiple myeloma. *Clin Chem* 2007;53:1099-1103

Kapoor P, Snozel CL, Colby C et al. Clinical impact of discordance in serum albumin measurements on multiple myeloma International Staging System. *J Clin Oncol* 2008;__:4051-4052

Rajkumar SV, Kyle RA, Therneau TM, et al. Serum free light ration is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood* 2005;106:812-817

Dispenzieri A, Kyle RA, Katzmann JA, et al. Immunoglobulin free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. *Blood* 2008;111:785-789

Riches PG, Sheldon J, Smith AM, et al. Overestimation of monoclonal immunoglobulin by immunochemical methods. *Ann Clin Biochem* 1991;18:253-259

Dingli D, Kyle RA, Rajkumar SV, et al. Immunoglobulin free light chains and solitary plasmacytoma of bone. *Blood* 2006;108:1979-1983

Salaun PY, Castinnet T, Frampas E, et al. FDG-positron-emission tomography for staging and therapeutic assessment in patients with plasmacytoma. *Haematologica* 2008;93:1269-1271

Table 1. Laboratory tests for multiple myeloma

- History and physical examination
- Complete blood count and differential; peripheral blood smear
- Chemistry screen including calcium and creatinine
- Serum protein electrophoresis, immunofixation
- Nephelometric quantification of serum immunoglobulins
- Routine urinalysis, 24 hour urine collection for electrophoresis and immunofixation
- Bone marrow aspirate and/or biopsy
- Cytogenetics (metaphase karyotype and FISH)
- Radiological skeletal bone survey including spine, pelvis, skull, humeri and femurs. Magnetic Resonance Imaging in certain circumstances
- Serum B2 microglobulin and lactate dehydrogenase
- Measurement of serum free light chains

