COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Specimens From Patients With Plasma Cell Neoplasms*

Version: Plasma Cell Neoplasms 1.0.0.2 **Protocol Posting Date:** January 2015
This protocol is NOT required for accreditation purposes

*This protocol applies to plasma cell neoplasms in bone marrow and extramedullary sites.

The following should NOT be reported using this protocol:

Monoclonal gammopathy of undetermined significance
Waldenström macroglobulinemia (lymphoplasmacytic lymphoma)
B-cell lymphomas with prominent plasmacytic differentiation (consider the non-Hodgkin lymphoma protocol)

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.

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Accreditation Requirements
This protocol can be utilized for clinical care purposes, but is not required for accreditation purposes.

CAP Laboratory Accreditation Program Protocol Required Use Date: Not applicable

CAP Plasma Cell Protocol Summary of Changes

V1.0.0.2
The following data element was modified:
Extent of Plasma Cell Infiltrate

V1.0.0.1
Revised document format

Surgical Pathology Cancer Case Summary

Protocol posting date: January 2015

PLASMA CELL NEOPLASM: Targeted Biopsy or Resection (Other Than Random Bone Marrow Sampling)

Note: This case summary is recommended for reporting plasma cell neoplasm specimens, but is not required for accreditation purposes.

Select a single response unless otherwise indicated.

Specimen (Note A)
___ Bone (specify site): _____
___ Nonosseous tissue (specify site): _____
___ Other (specify): _____
___ Not specified

Procedure
___ Biopsy
___ Resection
___ Fine-needle aspiration
___ Other (specify): _____
___ Not specified

+ Tumor Size (may be determined from radiographic studies)
+ Greatest dimension: ___ cm
+ Additional dimensions: ___ x ___ cm
+ ___ Cannot be determined

+ Clinical and Laboratory Data (select all that apply) (Note B)
+ ___ M-protein detected (specify type): _____
    + ___ Serum
    + ___ Urine
+ ___ Hypercalcemia
+ ___ Serum creatinine elevation
+ ___ Anemia
+ ___ Elevated serum beta-2-microglobulin (select one)
    + ___ <3.5 mg/L
    + ___ ≥3.5 mg/L to <5.5 mg/L
    + ___ ≥5.5 mg/L
+ ___ Serum albumin ≥3.5 g/dL
+ ___ Lytic bone lesions detected
+ ___ Other (specify): _____

+ Morphology (Note C)

+ Cytology
+ ___ Nonplasmablastic
+ ___ Plasmablastic

Immunoglobulin Deposits
___ Not detected
___ Amyloid
    ___ Congo Red stain
    ___ Subtyping performed (specify): _____
___ Nonamyloid (specify): _____
___ Not assessed

+ Subtype Based on the World Health Organization (WHO) Classification (Note D)
+ ___ Plasma cell myeloma
+ ___ Plasmacytoma
    + ___ Solitary plasmacytoma of bone
    + ___ Extraosseous plasmacytoma
    + ___ Plasmacytoma, not otherwise specified
+___ Other (specify): _____

Immunophenotype and Light Chain Type (based on immunohistochemistry, colorimetric in situ hybridization, and/or flow cytometry immunophenotyping) (Note E)

Immunoglobulin Light Chain
___ Kappa light chain
___ Lambda light chain
___ No light chain detected
___ Pending
___ Not performed
___ Other (specify): _____

+ CD19
+ ___ Not detected
+ ___ Detected

+ CD20
+ ___ Not detected
+ ___ Detected
    + Percentage of positive cells: _____ %

+ CD38
+ ___ Not detected
+ ___ Detected

+ CD56
+ ___ Not detected
+ ___ Detected

+ CD117 (KIT)
+ ___ Not detected
+ ___ Detected

+ CD138
+ ___ Not detected
+ ___ Detected

+ Cyclin D1
+ ___ Not detected
+ ___ Detected

+ Conventional Cytogenetics (Note F)
+ ___ Normal
+ ___ Abnormal (select all that apply)
    + ___ Complex (3 or more abnormalities)
    + ___ t(11;14)(q13;q32)
    + ___ -13
    + ___ del(17)(p13)
+ ___ No growth
+ ___ Not performed

+ Fluorescence In Situ Hybridization (Note F)

+ Monosomy 13/del(13q)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ del(17p)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ t(11;14)(q13;q32)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ *IGH* Gene (14q32) Rearrangement Other Than t(11;14)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ ___ Other probes (specify): _____

+ ___ Not performed

+ Comment(s)
_____
_____

Surgical Pathology Cancer Case Summary

Protocol posting date: January 2015

PLASMA CELL NEOPLASM: Bone Marrow Sample (Random, Nontargeted)

Note: This case summary is recommended for reporting plasma cell neoplasm specimens, but is not required for accreditation purposes.

Select a single response unless otherwise indicated.

+ Specimen
+ ___ Iliac crest
    + ___ Right
    + ___ Left
+ ___ Sternum

Procedure
___ Aspiration
    + ___ Satisfactory quality
    + ___ Suboptimal/unsatisfactory (specify): _____
___ Clot preparation
    + ___ Satisfactory quality
    + ___ Suboptimal/unsatisfactory (specify): _____
___ Core biopsy
    + ___ Satisfactory quality
    + ___ Suboptimal/unsatisfactory (specify): _____

+ Clinical and Laboratory Data (select all that apply) (Note B)
+ ___ M-protein detected (specify type): _____
    + ___ Serum
    + ___ Urine
+ ___ Hypercalcemia
+ ___ Serum creatinine elevation
+ ___ Anemia
+ ___ Elevated serum beta-2-microglobulin (select one)
    + ___ <3.5 mg/L
    + ___ ≥3.5 mg/L to <5.5 mg/L
    + ___ ≥5.5 mg/L
+ ___ Serum albumin ≥3.5 g/dL
+ ___ Lytic bone lesions detected
+ ___ Other (specify): _____

Morphology (Note C)

+ Extent of Plasma Cell Infiltrate in the Bone Marrow
+ Plasma cells on aspirate smear/touch preparation: ____ %
+ Plasma cells on core biopsy or clot: ____ %
    + ___ Estimate based on hematoxylin-and-eosin stain
    + ___ Estimate based on immunohistochemistry stain (eg, CD138)

+ Cytology
+ ___ Nonplasmablastic
+ ___ Plasmablastic

Immunoglobulin Deposits
___ Not detected
___ Amyloid
    ___ Congo Red stain
    ___ Subtyping performed (specify): _____
___ Nonamyloid (specify): _____
___ Not assessed

Subtype Based on the World Health Organization (WHO) Classification (Note D)
___ Plasma cell myeloma
___ Other (specify): _____

Immunophenotype and Light Chain Type (based on immunohistochemistry, colorimetric in situ hybridization, and/or flow cytometry immunophenotyping) (Note E)

Immunoglobulin Light Chain
___ Kappa light chain
___ Lambda light chain
___ No light chain detected
___ Pending
___ Not performed
___ Other (specify): _____

+ CD19
+ ___ Not detected
+ ___ Detected

+ CD20
+ ___ Not detected
+ ___ Detected
    + Percentage of positive cells: _____ %

+ CD38
+ ___ Not detected
+ ___ Detected

+ CD56
+ ___ Not detected
+ ___ Detected

+ CD117 (KIT)
+ ___ Not detected
+ ___ Detected

+ CD138
+ ___ Not detected
+ ___ Detected

+ Cyclin D1
+ ___ Not detected
+ ___ Detected

+ Conventional Cytogenetics (Note F)
+ ___ Normal
+ ___ Abnormal (select all that apply)
    + ___ Complex (3 or more abnormalities)
    + ___ t(11;14)(q13;q32)
    + ___ -13
    + ___ del(17)(p13)
+ ___ No growth
+ ___ Not performed

+ Fluorescence In Situ Hybridization (Note F)

+ Monosomy 13/del(13q)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ del(17p)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ t(11;14)(q13;q32)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ *IGH* Gene (14q32) Rearrangement Other Than t(11;14)
+ ___ Absent
+ ___ Present
+ ___ Not performed

+ ___ Other probes (specify): _____

+ ___ Not performed

+ Comment(s)
_____
_____

Explanatory Notes

Introduction
The purpose of this protocol is to report, in a synoptic format, the morphologic, immunophenotypic, and cytogenetic characteristics of a definitive initial diagnostic sample with a plasma cell neoplasm. It is not intended to serve as a practice guideline but rather as a reporting tool. It is recognized that many of the ancillary studies might not be available at the time of original diagnosis; thus, this protocol might be best completed at the time that all such results become available. The use of the bone marrow section of the protocol for follow-up bone marrow samples in patients with plasma cell myeloma is optional.

A. Specimen
The diagnosis of a plasma cell neoplasm can be made on a sample obtained from a lesion involving bone or an extraosseous site. Sample types include fine-needle aspiration, core biopsy, incisional biopsy, or excisional biopsy, all of which are acceptable for establishing a primary diagnosis in the proper clinical and imaging context and with proper immunophenotypic support. It should be noted that plasma cell neoplasms, particularly in extraosseous sites, should be distinguished from low-grade B-cell neoplasms with prominent plasmacytic differentiation. If the latter is a consideration, tissue sampling would be necessary for optimal diagnostic evaluation. In this protocol, reporting of extramedullary plasma cell neoplasms is kept separate from bone marrow involvement by monoclonal plasma cell proliferations on the empiric premise that bone marrow involvement is a manifestation of systemic disease.

B. Clinical and Laboratory Data
Plasmacytoma is a tumor of monoclonal plasma cells. A diagnosis of solitary plasmacytoma of bone can only be established by excluding other bone lesions by performing skeletal surveys and demonstrating the absence of monoclonal plasma cells in the bone marrow.[1] Most patients present with pain or a pathologic fracture. Extraosseous plasmacytoma is similarly a plasma cell tumor arising in tissues other than bone. While solitary plasmacytoma of bone is associated with a high risk of subsequent plasma cell myeloma, extraosseous plasmacytoma tends to be indolent with low risk of progression to plasma cell myeloma.

The diagnostic criteria for plasma cell myeloma include the triad of monoclonal bone marrow plasma cells, M-protein in the serum and/or urine, and evidence of related end-organ damage (hyper*c*alcemia, *r*enal insufficiency, *a*nemia, and/or *b*one lesions, abbreviated CRAB).[2] If end-organ damage is present, no minimum criteria for M-protein or monoclonal plasma cells are needed to make a diagnosis of symptomatic plasma cell myeloma. In the absence of end-organ damage, M-protein >30g/L and/or at least 10% monoclonal plasma cells in the bone marrow are needed to establish a diagnosis of asymptomatic/smoldering myeloma. Criteria for a diagnosis of plasma cell myeloma in patients with amyloidosis should be the same as for asymptomatic (smoldering) myeloma: a minimum of 10% bone marrow monoclonal plasma cells and/or M-protein at myeloma levels (>3g/dL of IgG or >2g/dL IgA). Unless these minimum criteria are met, the diagnosis should be primary amyloidosis, not plasma cell myeloma.

Plasma cell myeloma is a heterogeneous disease. While risk stratification schemes to guide therapeutic decisions have been broadly adopted, prognostication models continue to evolve as the biology of the disease is further elucidated and therapeutic approaches expand.[2-4] The Durie-Salmon staging system is used commonly and groups patients on the basis of the estimated tumor burden (Table 1).[5] Another system, the International Staging System (ISS) for plasma cell myeloma, is based on serum beta-2-microglobulin and albumin levels and is also widely used (Table 2).[6]

Table 1. Durie-Salmon Staging System for Plasma Cell Myeloma[5]*

Stage I	Low M-protein levels (IgG <50 g/L; IgA <30 g/L) Urine Bence-Jones protein <4 g/24 hours Absent or solitary bone lesions Normal hemoglobin, serum calcium, and non-M-protein Ig levels
Stage II	Overall values between stages I and III
Stage III	High M-protein levels (IgG >70 g/L; IgA >50 g/L) Urine Bence Jones protein >12 g/24 hours Multiple lytic bone lesions Hemoglobin <8.5 g/dL; serum calcium >12 mg/dL

* Patients are further subclassified based on renal function; A=serum creatinine <2 mg/dL; B=serum creatinine ≥2 mg/dL.
Abbreviation: Ig = immunoglobulin.
Note: Any one or more of the listed corresponding abnormalities would fulfill criteria for stage III.

Table 2. International Staging System for Plasma Cell Myeloma[6]

Stage I	Serum beta-2-microglobulin <3.5 mg/L; and, Serum albumin ≥3.5 g/dL
Stage II*	Not stage I or III
Stage III	Serum beta-2-microglobulin ≥5.5 mg/L

* Two stage II categories were identified: serum beta-2-microglobulin <3.5 mg/L, but serum albumin <3.5 d/dL and serum beta-2-microglobulin 3.5 to <5.5 mg/L regardless of serum albumin level.

C. Morphology

Neoplastic plasma cells have variable morphology. They range from low-grade, resembling normal plasma cells (Marshalko-type), to high-grade, characterized by high nucleus-to-cytoplasm ratio, irregular nuclear contours, open chromatin pattern, and prominent nucleoli. With the exception of plasmablastic morphology, grading of neoplastic plasma cells has not been identified as an independent prognostic parameter.

If amyloid deposits are suspected by routine microscopy, assessment using the Congo red stain with polarized light often offers confirmatory support.[7,8] However, amyloidosis is a heterogeneous disease caused by extracellular deposition of any one of 25 different proteins in insoluble beta-pleated fibrillar sheets. Since the management of patients with amyloidosis depends on the nature of the protein deposited, typing of amyloid deposits has become increasingly important. Amyloid typing may be performed using immunohistochemistry or mass spectrometry-based proteomics.[9,10]

In the bone marrow, assessment of the extent of involvement by neoplastic plasma cells is notoriously dependent on sample type. Accordingly, the percentages of plasma cells on Wright-Giemsa-stained bone marrow aspirate smears or touch preparations, as well as an estimate of the percentage of plasma cells in the bone marrow core biopsy or clot, are equally important. Estimating the number of plasma cells in the core biopsy or clot can be enhanced by using CD138 immunohistochemistry, particularly in samples with low to moderate involvement.

D. Histologic Type

This protocol is intended to be used for bona fide plasma cell neoplasms only, which include plasmacytoma, plasma cell myeloma (including asymptomatic/smoldering myeloma, nonsecretory myeloma, and plasma cell leukemia), and immunoglobulin deposition diseases listed below (Table 3). The use of this reporting protocol is not intended for monoclonal gammopathy of unknown significance (MGUS), which is not considered a neoplastic process in the current World Health Organization (WHO) classification.[1] Furthermore, this protocol is not intended to be used for cases of B-cell lymphoma with prominent plasmacytic differentiation.

Table 3. World Health Organization Classification of Plasma Cell Neoplasms[1]

Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases
Primary amyloidosis
Systemic light and heavy chain deposition diseases

E. Immunophenotype and Light Chain Type

Immunophenotyping of plasma cell neoplasms can be performed using immunohistochemistry and/or flow cytometry immunophenotyping. Neoplastic plasma cells are generally positive for CD38 and CD138, dim for CD45, and negative for CD19; in addition, CD20, CD56, and CD117 expression may be present in subsets of cases.[11,12] Constitutive overexpression of cyclin D1 is generally associated with the presence of t(11;14) or other genomic mechanisms such as polysomy of chromosome 11.[13,14] The immunostains listed in this protocol are not meant as practice guidelines. In addition to immunohistochemistry and flow cytometry, light chain expression is increasingly being assessed using colorimetric in situ hybridization.

F. Cytogenetic and Fluorescence In Situ Hybridization Studies

Cytogenetic abnormalities are common in plasma cell myeloma. Conventional cytogenetic analysis demonstrates an abnormal karyotype in up to 30% of cases, and such patients have more adverse outcomes compared to those with diploid karyotype by cytogenetics. Cytogenetic abnormalities are broadly categorized as hyperdiploid and nonhyperdiploid, with the former being generally associated with better outcomes.[15,16] Hyperdiploid cases commonly have extra copies of odd-numbered chromosomes. Plasma cell neoplasms with nonhyperdiploid cytogenetics are more likely to harbor nonrandom balanced chromosomal translocations involving the immunoglobulin heavy chain gene, *IGH*, at chromosome 14q32. With the exception of t(11;14)(q13;q32), most such translocations are associated with adverse outcomes.[4]

Neoplastic plasma cells often grow poorly in cell culture; therefore, fluorescence in situ hybridization (FISH) is commonly used to enhance the sensitivity of detecting cytogenetic abnormalities in plasma cell myeloma and plays an important role in prognostic assessment.[17,18] While interphase FISH can be performed on unenriched bone marrow aspirate material, its sensitivity can be improved by plasma cell enrichment or through cytoplasmic immunoglobulin-enhanced FISH.[19] The International Myeloma Working Group has provided recommendations for the minimum baseline genetic information that needs to be obtained in all cases of plasma cell myeloma, as outlined in Table 4.[20] Although the independent prognostic implications of chromosome 13 abnormalities are in question, most laboratories continue to screen for -13/del(13q), as positive results are regarded as an adjunct data point that, in combination with other variables such as beta-2-microglobulin, can help identify patients with high-risk disease.[17,18,20]

Table 4. Plasma Cell Myeloma Fluorescence In Situ Hybridization Clinical Testing Recommendations by the International Myeloma Working Group[20]

Minimum Panel	t(4;14)(p16;q32) t(14;16)(q32;q23) del(17p13)
Comprehensive Panel	t(11;14)(q13;q32) -13 or del(13q) Ploidy category Chromosome 1 abnormalities

Using conventional cytogenetics or FISH, patients with plasma cell myeloma can be broadly risk-stratified as outlined in Table 5.[17,18]

Table 5. Plasma Cell Myeloma Cytogenetic Risk Groups

Unfavorable	Aneuploid or hypodiploid karyotype -13 or del(13q) t(4;14)(p16;q32) t(14;16)(q32;q23) del(17p13)
Favorable	Hyperdiploid karyotype t(11;14)(q13;q32)

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