

# Communiqué

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A M a y o R e f e r e n c e S e r v i c e s P u b l i c a t i o n

 REFERENCE LABORATORY

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## Polycythemia Vera

Polycythemia vera (PV) is a somewhat rare (approximately 2/100,000 persons/year<sup>1</sup>) chronic myeloproliferative disease (CMPD). CMPDs cause an abnormal production of the various types of blood cells. PV, also known as erythrocythemia or primary polycythemia, is characterized by autonomous production of hematopoietic cells. The disease primarily causes an overproduction of red blood cells (erythrocytes), but also may cause an increase in white blood cells or platelets. Of these, the overproduction of red blood cells (RBC) has the greatest impact on the patient's health. As the RBC level rises, blood viscosity increases, which combined with the possibly high number of platelets, increases the risk for clot formation, stroke, heart attack, and bleeding. Patients with PV also are at increased risk for transformation to myeloid metaplasia or acute leukemia.<sup>1</sup> While there is no cure for PV, treatments are available that can significantly reduce the risk for complications. As a result, when patients present with an unexplained increase in hematocrit or hemoglobin value, the physician needs to rule out the possibility of PV.

### Other Forms of Polycythemia

There are other forms of polycythemia. These forms should be considered in the differential diagnosis but often are relatively easy to distinguish from PV. Relative polycythemia is a condition that may not require treatment and is a result of a relative increase in red cell mass (RCM) due to a decrease in plasma volume levels.<sup>1</sup>

Secondary polycythemia exists in 2 forms: acquired and congenital. The acquired form of the disease is associated with an erythropoietin (EPO) increase that is either hypoxia driven (eg, seen in patients living at high altitudes or with pulmonary disease), or oxygen independent (eg, caused by the presence of a tumor).<sup>2</sup> In the congenital form of secondary polycythemia, serum EPO levels may be normal or elevated.

EPO is a large (193 amino acid residue) glycoprotein hormone that is secreted by the kidney and regulates RBC production. Hypoxia (abnormally low levels of oxygen in the blood) stimulates EPO release, which in turn stimulates bone marrow erythrocyte production. High levels of RBC, hemoglobin, hematocrit, or oxygen suppress the release of EPO. Congenital PV cases are typically due to the presence of an abnormal hemoglobin with a high oxygen affinity. A diagnosis of congenital secondary polycythemia should be suspected when there is either a family history of such or the disease presents at a young age.<sup>1</sup> Finally, there is "inapparent polycythemia" in the patient with an increased RCM who has an apparently normal hematocrit because of an increased plasma volume. Usually, these patients are discovered because of a PV-related feature, rather than a finding on a complete blood count (CBC).

### Clinical Features of Polycythemia Vera

The usual PV patient is over 60 years of age, although the disease may present as early as childhood, with slightly more men than women developing the disease.<sup>1</sup> They may exhibit a flushed or ruddy appearance. While some patients with PV may experience no symptoms, findings that are commonly seen in PV include:

#### Clinical

- Hyperviscosity symptoms: headaches, dizziness, visual symptoms, paresthesias, fatigue<sup>1</sup>
- Splenomegaly: abdominal discomfort, weight loss, night sweats<sup>1</sup>
- Unusual thrombotic history
- Post-bath pruritus (itching)
- Retinal vein distension<sup>1</sup>
- Erythromelalgia (pain, throbbing, burning in one or more extremities)
- Budd-Chiari syndrome
- Digital ischemia

#### Laboratory

- Persistent leukocytosis
- Persistent thrombocytosis
- Microcytosis due to iron deficiency

- Bone marrow panhyperplasia with atypical megakaryocytes
- Bone marrow reticulin fibrosis
- Clonal cytogenetic abnormalities
- In vitro formation of endogenous erythroid colonies (EEC)

With individual variation in hemoglobin levels (males: 13.5-17.5 g/dL; females: 12-15.5 g/dL), there should be a thorough review of the patient's medical history to document whether the elevated level is a change from the patient's baseline. Collecting a detailed medical history from the patient can further aid the physician by identifying clinical characteristics that support the diagnosis.

It is important to note that patients with PV who are iron deficient (eg, as a result of gastrointestinal or other bleeding) may have pseudonormal RBC levels. For this reason, when patients present with unexplained thrombocytosis or leukocytosis, PV needs to be considered in the differential diagnosis.

### Diagnosis

Developed by the Polycythemia Vera Study Group<sup>3</sup>, the criteria for a diagnosis of PV are broken into 2 classifications: major and minor.<sup>1</sup> The major criteria include demonstration of increased RCM, splenomegaly, and normal arterial oxygen saturation. The presence of all 3 major criteria is diagnostic for PV. The minor criteria include features of pluripotential precursor cell involvement, such as increased platelet counts or neutrophil counts, increased leukocyte alkaline phosphate activity, or increased vitamin B12 binding protein. The presence of increased RCM and normal arterial oxygen saturation combined with any 2 of the minor criteria is diagnostic for PV. However, the RCM has limitations, both in its performance and in its detection of early disease.

An alternative diagnostic approach has been proposed, utilizing EPO and the culture of an endogenous erythroid colony (EEC) (see Figure 1).<sup>4</sup> Increased erythrocytes result in compensatory suppression of EPO levels. Therefore, low EPO levels in conjunction with abnormally high RBC levels is highly suggestive of PV. Among the causes of erythrocytosis, only PV results in below-normal serum EPO levels.<sup>4</sup> The development of an EEC in the presence of below-normal serum EPO levels is specific for PV. However, the EEC assay is not widely available and requires a high level of expertise to perform.<sup>4</sup>

### Laboratory Testing for Polycythemia Vera

Numerous tests may be utilized to reach a diagnosis. The following tests are grouped as either initial or advanced studies.

#### Initial Studies

##### Complete Blood Count

Initial testing should include a CBC with differential. A hemoglobin level of >18.5 g/dL in men or >16.5 g/dL in women is suggestive of PV, likewise with a hemocrit level of > 60% in men or 53% in women.<sup>2</sup>

##### Erythropoietin

When a patient is suspected of having PV, the serum EPO level should be checked, [#80173 Erythropoietin \(EPO\), Serum](#). In the appropriate clinical setting, PV is likely when EPO levels are suppressed, but unlikely when EPO levels are elevated. However, EPO levels alone cannot reliably distinguish between low-normal and suppressed EPO levels; EPO levels are within normal limits in some patients with primary polycythemia.

##### Leukocyte Alkaline Phosphatase

Leukocyte alkaline phosphatase (LAP) ([#9699 Leukocyte Alkaline Phosphatase \(LAP\) Score](#)) is normally present in the secondary granules of mature neutrophils. LAP activity in neutrophils may change in various diseases. Increased LAP activity is usually seen in PV and reactive neutrophilia. LAP level >100 is one of the minor criteria for a diagnosis of PV.

##### Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub> (cobalamin) is necessary for hematopoiesis. In PV, an elevated vitamin B12 level occurs secondary to an increase in transcobalamin III, which is produced by the granulocytes (nonspecific effect of the myeloproliferation). [#9154 Vitamin B-12 Assay, Serum](#) may be utilized when an LAP measurement is normal or unavailable.

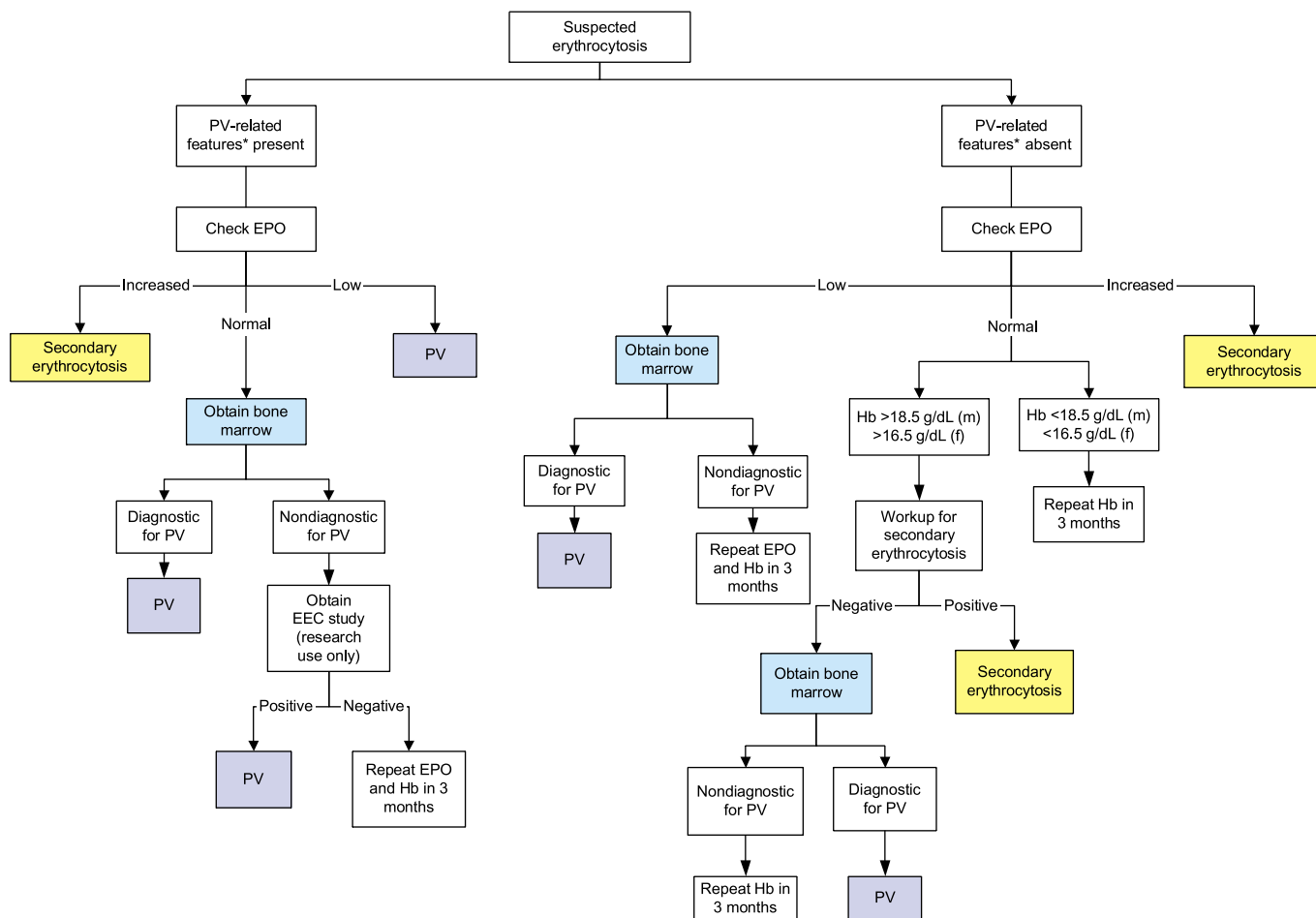
##### Chemistry Tests

Several routine chemistry tests can assist in establishing a diagnosis: sodium, potassium, creatinine, blood urea nitrogen (BUN), alkaline phosphatase, aspartate aminotransferase (AST), and bilirubin.

#### Advanced Studies

##### Bone Marrow Biopsy

When a working diagnosis of PV has been established, a bone marrow examination should be performed, [#9172 Bone Marrow Biopsy](#) or [#5434 Hematopathology Consultation](#).<sup>4</sup>



\*PV-related features include portal vein thrombosis, Budd-Chiari syndrome, splenomegaly, erythromelalgia, persistent leukocytosis, thrombocytosis, or microcytosis, post-bath pruritus, and digital ischemia.

**Figure 1. Diagnostic approach to suspected erythrocytosis.**

Modified from Tefferi A: *Myeloproliferative Disorders: The patient with erythrocytosis.* In *Primary Hematology*, Humana Press, Totowa, NJ, 2001, p 122. By permission of Mayo Foundation for Medical Education and Research.

### *Cytogenetic Studies*

Mayo hematopathologists routinely utilize evaluation of the bone marrow by cytogenetic studies, #8506 [Chromosome Analysis, For Hematologic Disorders, Bone Marrow](#), as several chromosome abnormalities have been associated with PV including deletion 20, +8, and +9. Cytogenetic analysis may also include #80578 [Fluorescence In Situ Hybridization DNA Probes \(D-FISH\) with BCR/ABL](#) to rule out chronic myeloid leukemia in patients with thrombocytosis.

### *Red Blood Cell Mass*

A RCM  $\geq 36$  mL/kg for males and  $\geq 32$  mL/kg for females is one of the major criteria for PV. This test involves treating a patient's blood specimen with radioactive material and reinjecting it into the patient. The results provide an indirect estimate of the RCM. This test is not routinely utilized at Mayo and is not offered through MML. The RCM is unable to differentiate between PV and secondary polycythemia.<sup>4</sup>

### *Endogenous Erythroid Colony*

In this test, blood cells from a patient specimen are mixed with medium that does not contain EPO. The mixture is plated out and incubated. Plates are then evaluated for EEC formation. Non-EPO-stimulated cultures from normal patients will exhibit no EEC formation. Further studies will need to verify the utility of this test for routine diagnostic purposes. EEC testing is currently utilized only as a research test at Mayo.

### *Oxygen Dissociation P<sub>50</sub>*

The oxygen dissociation assay (#9110 [Oxygen Dissociation P50, Erythrocytes](#)) determines the oxygen-affinity of hemoglobin. An increase is reflected in a low P<sub>50</sub>, left-shifted curve, and loss of normal sigmoidal configuration and is characteristic of many hemoglobin variants that are responsible for congenital polycythemia. It is **not useful for the detection of polycythemia vera**, but is useful for the exclusion of a high-affinity hemoglobin as the cause of erythrocytosis.

### **Treatment**

Treatment for PV remains focused on phlebotomy. Removal of blood is performed on a daily or weekly schedule until target hematocrit levels are reached: <45% in men and <42% in women.<sup>1</sup> Once the target hematocrit level is reached, frequency of the phlebotomy is adjusted to maintain the target level. In patients who are at risk for thrombosis, additional therapy may be required. This group includes patients older than 60 years of age and those with a history of thrombosis. Chemotherapy is an option for these

patients. The chemotherapy most frequently utilizes hydroxyurea, given orally several times a day. The goal of hydroxyurea therapy is to keep platelet levels <400,000/ $\mu$ L, while maintaining leukocyte levels of >3,000/ $\mu$ L.

Treatment with high-dose aspirin should be avoided. While the aspirin provides antiplatelet therapy and reduces the risk of thrombosis, it increases the risk of gastric bleeding. Low-dose aspirin (81 mg/day) has a decreased risk of gastric bleeding and can be used to treat vasomotor symptoms.<sup>1</sup>

Finally, patients with PV should avoid behaviors that may increase their risk of thrombosis. This includes smoking (smoking constricts blood vessels and can increase the chance of stroke), sedentary lifestyle (patients should take regular rest periods, but also should perform light exercise like walking to improve circulation, and leg and ankle exercises to reduce the risk of clot formation), and a high sodium diet (sodium increases fluid retention, which may impair circulation).

For personal comfort, patients can reduce the post-bath pruritus of PV by bathing in cool water and patting, not rubbing, the skin dry. Use of lotions to keep skin moisturized can also reduce the skin irritation associated with PV. In addition, patients should inspect their feet on a regular basis for sores that may result from circulatory problems.

### **Conclusion**

A diagnosis of PV should be based primarily on the clinical presentation and patient history.<sup>4</sup> Laboratory findings alone cannot diagnose PV and must be correlated with patient information. Once diagnosed, patients with PV can undergo treatment to minimize their risk for the serious, life-threatening complications that accompany PV.

### **References**

1. Tefferi A: Chronic Myeloproliferative Diseases. *In* Primary Hematology. Edited by A Tefferi. Totowa, NJ, Humana Press, 2001, pp 119-148
2. Tefferi A, Silverstein MN: Myeloproliferative Diseases. *In* Cecil Textbook of Medicine, 21st edition. Edited by L Goldman. WB Saunders Co, 2000, pp 935-941
3. Wasserman LR: The Treatment of Polycythemia. A Panel Discussion. Presented at the Annual Meeting of the American Society of Hematology, Toronto, Ontario, Canada. Blood 1968;32(3):483-488
4. Tefferi A: Diagnosing Polycythemia Vera: A Paradigm Shift. Mayo Clin Proc 1999;74(2):159-162

**FISH for APL Method and Reference Value Change**  
[#80774 Fluorescence In Situ Hybridization \(FISH\) With 15;17 Translocation Probe for Acute Promyelocytic Leukemia \(APL\), Bone Marrow](#) has been converted to a D-FISH method. D-FISH involves the use of fluorescence in situ hybridization and DNA probes that detect *double fusion products*. This method detects PML/RAR $\alpha$  fusion, or variants involving RAR $\alpha$ , in nonproliferating nuclei (interphase cells). The D-FISH method detects PML/RAR $\alpha$  fusion on both the abnormal chromosome 15 and 17, and offers improved performance by detecting variants involving a breakpoint of only RAR $\alpha$ . This test involves scoring of 500 nuclei. The results are expressed as percent abnormal nuclei. The D-FISH method can detect disease when the translocation is present in as few as 0.6% of cells.

#### New Reference Values

An interpretive report is provided.

#### Previous Reference Values

Less than or equal to 2.5% of 200 interphase cells with fusion of PML and RAR $\alpha$  is consistent with a normal result. The normal range for the signal pattern associated with variant APL translocations involving RAR $\alpha$  only has not been established. Normal ranges were determined by using the binomial distribution and the upper bound of a 1-sided 95% confidence interval for observing the maximum number of abnormal nuclei in 200 and from 20 normal bone marrow transplant donor specimens.

#### New CPT Codes:

88237/Tissue culture for neoplastic disorders  
 88271x2/DNA probe, each  
 88275x2/Interphase in situ hybridization  
 88291/Interpretation and report

#### Previous CPT Codes:

88271x2/DNA probe, each  
 88275/Interphase in situ hybridization  
 88291/Interpretation and report

#### Myeloperoxidase Antibodies Reference Value Change

The reference values for [#80389 Myeloperoxidase Antibodies, Serum](#) changed in response to a change in the test kit. The expanded reference values include an equivocal range.

#### New Reference Values

≤5.0 EU/mL	Negative
5.1-14.9 EU/mL	Equivocal
≥15.0 EU/mL	Positive

#### Previous Reference Values

≤5.0 EU/mL	Negative
5.1-400.0 EU/mL	Positive

#### Insulin Antibodies Reporting Change

[#8666 Insulin Antibodies, Serum](#) has been changed and no longer detects or reports the presence of pork or beef insulin antibodies. This test only detects and reports human insulin antibodies.

#### Phospholipid Antibodies (Cardiolipin Antibodies)

##### Test Changes

The method for phospholipid antibodies was changed to a new kit, resulting in changes to the reference values for all 3 components. [#82976 Phospholipid Antibodies \(Cardiolipin Antibodies\) IgG and IgM, Serum](#) includes 2 components: [#80993 Phospholipid Antibodies \(Cardiolipin Antibodies\) IgG, Serum](#) and [#81900 Phospholipid Antibodies \(Cardiolipin Antibodies\), IgM, Serum](#). The reference value changes will apply in both the individual tests and the combined test.

#### New Reference Values

[#80993 Phospholipid Antibodies \(Cardiolipin Antibodies\), IgG, Serum](#)

≤15.0 GPL	Negative
15.1 – 79.9 GPL	Positive
≥80.0 GPL	Strongly positive

[#81900 Phospholipid Antibodies \(Cardiolipin Antibodies\), IgM, Serum](#)

≤10.0 MPL	Negative
10.1-79.9 MPL	Positive
≥80.0 MPL	Strongly positive

*GPL and MPL units are widely used to express the results of antiphospholipid antibody tests performed by enzyme immunoassays. The terminology refers to arbitrary units, and the abbreviations GPL and MPL denote whether the result is for the IgG or the IgM isotype, respectively. The letters PL denote specificity for phospholipid antigens.*

#### Previous Reference Values

[#80993 Phospholipid Antibodies \(Cardiolipin Antibodies\), IgG, Serum](#)

≤15.0 GPL	Negative
15.0–29.9 GPL	Weakly positive
30.0-79.9 GPL	Positive
>80.0 GPL	Strongly positive

[#81900 Phospholipid Antibodies \(Cardiolipin Antibodies\), IgM, Serum](#)

<15.0 MPL	Negative
15.0-29.9 MPL	Weakly positive
30.0-79.9 MPL	Positive
>80.0 MPL	Strongly positive

## Mayo Reference Services Celebrates National Laboratory Week – April 20-26, 2003 *Laboratory Professionals: Exceptional People – Exceptional Work*

Mayo Reference Services would like to take this opportunity to recognize and honor the dedication of medical laboratory professionals and board-certified pathologists. Their commitment to excellence in medical laboratory testing and interpretation is essential for the delivery of quality patient care.

Ask



US

**Q:** Does Mayo offer a polymerase chain reaction (PCR) test for *Mycobacterium tuberculosis*?

**A:** Mayo's amplified direct test ([#81383 Mycobacterium Tb, Amplified Direct Test](#)) uses a PCR-like method known as transcription-mediated amplification (TMA). In this test, a specific mycobacterial rRNA target is amplified under isothermal conditions using DNA intermediates. The amplified RNA target is then detected using a sequence-specific chemiluminescent hybridization probe. This test detects members of the *M tuberculosis* complex (which include *M tuberculosis*, *M bovis*, *M bovis (bacille Calmette-Guérin)*, *M africanum*, *M microti*, and *M canetti*). In rare instances, tests for *M tuberculosis* may cross-react with *M celatum* and *M terrae*-like organisms, which are infrequent but proven human pathogens.

**Q:** How does the Amplified Direct test ([#81383](#)) compare with traditional PCR?

**A:** The Amplified Direct test offers several advantages over traditional PCR. The presence of multiple rRNA copies in each mycobacterium organism provides increased sensitivity compared with traditional DNA amplification by PCR. The Amplified Direct test performed by Mayo is the only commercially available method approved by the FDA for use with both acid-fast smear-positive and smear-negative specimens. In addition, the test has improved detection rates for nonrespiratory specimens compared with current commercially available PCR tests for *M tuberculosis*. Accordingly, [#81383 Mycobacterium Tb, Amplified Direct Test](#) is MML's preferred test for direct detection of mycobacterial RNA.

*Reference: O'Sullivan CE, Miller DR, Schneider PS, and Roberts GD: Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test by Using Respiratory and Nonrespiratory Specimens in a Tertiary Care Center Laboratory. J Clin Microbiol 2002;40:1723:1727*

# 2003 Meeting Calendar

## *Interactive Satellite Programs . . .*

## *Upcoming Education Conferences . . .*

### *Systemic Lupus Erythematosus Diagnosis and Treatment*

April 10, 2003

Presenters: Shreyasee Amin, MD & Kevin Moder, MD  
Moderator: Steven Ytterberg, MD

### *The Many Faces of Juvenile Rheumatic Disease*

May 13, 2003

Presenters: Thomas Mason, MD & Ann Reed, MD  
Moderator: Steven Ytterberg, MD

### *Clinical Presentation and Laboratory Diagnosis on Agents of Bioterrorism*

June 3, 2003

Presenters: Franklin Cockerill III, MD,  
Thomas F. Smith, PhD, & Joseph Yao, MD  
Moderator: Robert Kisabeth, MD

### *Seronegative Spondyloarthropathies: Review and Recent Advances*

September 16, 2003

Presenters: Nisha Manek, MD & Clement Michet, Jr, MD  
Moderator: Steven Ytterberg, MD

### *Pharmacogenetics and Pharmacogenomics of Antidepressants*

October 7, 2003

Presenters: John Black, MD  
David Mrazek, MD  
Elliott Richelson, MD  
Moderator: Robert Kisabeth, MD

### *Pharmacogenomics of Warfarin Therapy*

November 11, 2003

Presenter: John Height, MD  
Moderator: Robert Kisabeth, MD

### *Cardiac Markers*

December 2, 2003

Presenter: Allan Jaffee, MD  
Moderator: Robert Kisabeth, MD

### *Practical Spirometry*

April 29-30, 2003

Radisson Hotel  
Rochester, Minnesota

### *Quality Issues in Phlebotomy*

April 10-11, 2003

Mayo Clinic, Siebens Building  
Rochester, MN

### *Integration Through Community Laboratory Insourcing: Implementing a Successful Laboratory Program*

October 8-10, 2003

Providence Marriott Hotel  
Providence, Rhode Island

### *International Surgical Pathology Symposium*

May 6-9, 2003

Four Seasons Hotel  
Dublin, Ireland

### *Practical Surgical Pathology*

September 11-13, 2003

Mayo Clinic, Siebens Building  
Rochester, Minnesota



For a complete listing of all the courses offered throughout the year, contact the Mayo Reference Services Education Office at 1-800-533-1710 or 507-284-8742.

# Abstracts of Interest

## Review of 1027 Patients With Newly Diagnosed Multiple Myeloma

Robert A. Kyle, MD; Morie A. Gertz, MD; Thomas E. Witzig, MD; John A. Lust, MD, PhD; Martha Q. Lacy, MD; Angela Dispenzieri, MD; Rafael Fonseca, MD; S. Vincent Rajkumar, MD; Janice R. Offord, BS; Dirk R. Larson, MS; Matthew E. Plevak, BS; Terry M. Therneau, PhD; and Philip R. Greipp, MD

- **Objective:** To determine the clinical and laboratory features of newly diagnosed multiple myeloma.
- **Patients and Methods:** Records of all patients in whom multiple myeloma was initially diagnosed at the Mayo Clinic in Rochester, Minn, from January 1, 1985, to December 31, 1998, were reviewed.
- **Results:** Of the 1027 study patients, 2% were younger than 40 years, and 38% were 70 years or older. The median age was 66 years. Anemia was present initially in 73% of patients, hypercalcemia (calcium level  $\geq 11$  mg/dL) in 13%, and a serum creatinine level of 2 mg/dL or more in 19%. The  $\beta_2$ -microglobulin level was increased in 75%. Serum protein electrophoresis revealed a localized band in 82% of patients, and immunoelectrophoresis or immunofixation showed a monoclonal protein in 93%. A monoclonal light chain was found in the urine in 78%. Non-secretory myeloma was recognized in 3% of patients, whereas light-chain myeloma was present in 20%. Conventional radiographs showed an abnormality in 79%. The plasma cell labeling index was 1% or more in 34% of patients. Multivariate analysis revealed that age, plasma cell labeling index, low platelet count, serum albumin value, and the log of the creatinine value were the most important prognostic factors.
- **Conclusion:** The median duration of survival was 33 months and did not improve from 1985 through 1998.

Mayo Clinic Proceedings 2003;78:21-33

The complete article is available on-line at URL: <http://www.mayo.edu/proceedings/>

## Communiqué

[www.mayoreferenceservices.org/communique/](http://www.mayoreferenceservices.org/communique/)

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