TULIP NEWS

Tulip Group Does it again with its excellent Anti-A1 lectin and Anti-H lectin reagents

In the last issue we had discussed our interest in assisting customers in identifying and solving blood grouping related issues. Last week we came across another interesting case of a rare blood group sample which we would like to share with you.

Case history

Patient Information: Male, 54 years
Patient ID: (AHB/7410).
History: No previous history of transfusion, patient having cardiac problem.
Other information:
(a) Patient's blood sample is showing as weak 'A' positive in forward grouping (1+ reaction). Reacts strongly (4+) with B group cells in reverse grouping
Nature of the query: Identification of blood group of the patient sample

Further to this at Tulip we carried the following tests
Note: In the first 3 evaluations known A1 Rh +ve blood group was used as a control

1. Forward grouping ABD (Tube test)

Reagent	Lot. No	Patient's sample	known A1 group
Eryscreen Anti-A	124627	1+	4+
Eryscreen Anti-B	124627	-	-
Eryscreen Anti-D	124627	3+	3+

Observations:
1.Patient's blood sample showed weak reaction (1+) with Anti-A reagent as compared to known A1 group sample (4+ reaction).
2.Both patient's sample and known A1 group sample gave strong reaction (3+) with Anti-D (IgM).
Patient's blood sample could be weak A Rh +ve group

2. Tube test with Erybank Anti- A1 lectin

Reagent	Lot. No	Patient's sample	known A1 group
Erybank A1 lectin	1077021	-	3+

Observations:
Patient's blood sample showed no reaction with Anti-A1 lectin reagent whereas known A1 group sample presented a 3+ reaction. Therefore patient's sample is not A1 (stronger subgroup of A) group. It could be a weaker subgroup of A (i.e.,A2, A3, Ax etc)

3. Reverse grouping of the serum sample with known cells

Known cells	Patient's serum sample	known A1 group serum
A1 Rh +ve	-	-
B Rh +ve	4+	4+

Observations:
Both Patient's serum sample & known A1 serum sample showed no reaction with A1 Rh +ve cells and strong 4+ reaction with B Rh +ve cells, as expected of A group sample.

4. Tube test with Erybank Anti- H lectin

Reagent	Lot. No	Patient's sample	known A2 group	known A1 group
Anti-H lectin	1077021	3	2	±

Observations:
Comparative test of patient sample, known A1 group sample and known A2 group sample with
Anti-H lectin was done, as Anti-H lectin is known to exhibit different reactivity pattern with these cells (H as we know is precursor antigen, In A1 group, A antigenic sites is more, H is less, In A2 group A antigenic sites is less, H is more, In A3, A is still less, H is more than in A2 subjects).From the above evaluation the reactivity exhibited by the patient's sample was more than A1 or A2 group sample. Therefore the sample could be of A3 group. Further one more evaluation test for confirming A3 group was conducted using the serum of known O group and B group person as mentioned in Blood Transfusion in Clinical Medicine By P.L. Mollison, 10 th Edition.

5. Test patient cells with known O and B group serum

Serum	Patient cells
known O group (contains A, B antibodies)	2+
known B group (contains A antibodies)	±

Observations:
The above test result indicates that the patient's blood sample could be A3 Rh +ve group

ERYBANK Anti-H lectin ------ ERYBANK Anti -A1 lectin

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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

It is over three years since we commenced the publication of The Crux, a number of diseases and hot spots have been amply elucidated and laid thread bare for easier understanding and assimilation.

We started with topics of our choice and then came a barrage of requests for topics of then current or contemporary significance. Each issue has interpreted something and likewise every single issue has trouble shot one or the other day-to-day routinely encountered problem for you. If we could gather all articles within a single cover, it would assemble into a very useful book - a sort of a "COLLECTOR'S ITEM" as far as laboratarians are concerned.

Of course, it has not been all work and no play; we have supplied some laughing gas and tickling fingers too along with each issue.

A request came a couple of months back to consider in detail a not so uncommonly encountered neoplastic disease called as Plasmacytoma/ Multiple Myeloma.

The DISEASE DIAGNOSIS section discusses Multiple Myeloma at length; all clinico-diagnostic aspects have been presented. In order to keep the details adequate for correct understanding of the disorder we could not cut short the size of the text involved. Therefore the proper classification and staging parameters have been taken under the ambit of INTERPRETATION segment.

As Pregnancy and related Immunohaematolgy could be not be covered completely in the last issue, the same has been carried over to completion in this issue. It is hoped that hereafter the number of HDN cases would drop to extremely minimal levels. TROUBLE SHOOTING shall clear up all haze that we encounter when handling HDN cases.

Half a page has been strewn with various colours of wisdom, laughter and knowledge. BOUQUET has not been overlooked.

Between the covers, every word shall ooze sense when squeezed. So just go ahead right now, right away.

Tulip Group

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DISEASE DIAGNOSIS

MULTIPLE MYELOMA

Description

Neoplasm of mature and immature plasma cells. Classically involves the skeleton. Symptoms result from marrow replacement, bone destruction, and production of monoclonal antibodies or antibody fragments. Typically presents with recurrent bacterial infections, anemia, osteolytic lesions, and renal insufficiency. Chemotherapy is standard treatment but is not curative. Roles of allogeneic or autologous bone marrow or peripheral blood stem cell rescue are promising and are still being evaluated

Synonyms

Plasma cell myeloma, Myeloma, Myelomatosis, Kahler's disease

Background

Cardinal features

Malignancy of single plasma cell line. Presents with an excessive production of monoclonal immunoglobulins by malignant cells, which appears as an M-spike (or monoclonal band) in serum protein electrophoresis. Osteolytic lesions are caused by the rapid growth of the malignant plasma cells and by excessive osteoclast activation, and they result in bone pain, pathologic fractures, and hypercalcemia. Usually affects multiple bony sites, High monoclonal antibody levels cause hyperviscosity syndromes: blurred vision, dizziness, and altered consciousness. 55% produce immunoglobulin (Ig) G paraprotein, 21% IgA, 22% light chain only, and 2% others (IgD, IgE). A small number of malignant plasma cells are found in the circulation but most are in the bone marrow. Light chain fragments may be excreted in the urine as Bence Jones protein (found in 75%). Bone marrow replacement causes anemia, thrombocytopenia, and immunosuppression leading to fatigue, bruising, and chronic infections. Monoclonal antibody fragments and hypercalcemia, as much as secondary amyloidosis, may cause chronic renal failure

Causes

Common causes

Etiology usually unknown.

Contributory or predisposing factors

Predisposing factors usually unknown. Because peak age is among the elderly it is thought that susceptibility may increase with the aging process, with reduction in immune surveillance, and a life-long accumulation of toxic insults or antigenic challenges. Agricultural workers, petroleum workers, workers in leather industries, and cosmetologists with exposure to herbicides, insecticides, petroleum products, heavy metals, plastics, and various dusts including asbestos have been considered to be potentially at an increased risk developing multiple myeloma, although the risk is ill-defined and has not been quantified. People exposed to large amounts of radiation (such as survivors of the atomic bomb explosions in Japan) have an increased risk. Human herpes virus 8 (HHV-8), a new human herpes virus, has been found in the nonmalignant bone marrow dendritic cells of patients with myeloma. The role of HHV-8 needs to be determined. May be preceded by solitary plasmacytoma or monoclonal gammopathy of undetermined significance (MGUS) - a significant percentage of these patients go on to develop myeloma.

Epidemiology

Incidence

Annual incidence: 4 per 100,000. The apparent increase in incidence is probably related to the increased availability and use of medical facilities, and to better diagnostic techniques

Demographics

Age

Predominantly age 40-80 years. Peak incidence in seventh decade. Median age: 69 years (men), 71 years (women). Only 18% of sufferers are < 50 years. Only 3% of sufferers are < 40 years

Gender

More commonly affects males (1.5 times).

Genetics

Abnormal karyotypes are present in 30-40% of patients but there is no specific karyotypic abnormality. Chromosome abnormalities are present in about 50% of patients. Monosomy 13 and trisomy or tetrasomy for chromosome 9 are the most common numerical abnormalities. Poor prognosis: loss of 13, translocation of 11q, translocation of 1q. Good prognosis: trisomy of 6, 9, and 17. Slight increased risk among children and siblings of those with myeloma

Diagnosis

Clinical presentation

Symptoms

20% of patients are asymptomatic at diagnosis and picked up by blood test. Bone symptoms - bone pain, typically in the back or thorax and described as wandering and intermittent, in 60% at presentation. Symptoms caused by bone marrow infiltration symptoms - fatigue or weakness, palpitations, dyspnea, and poor wound healing due to normocytic, normochromic anemia (60% are anemic at presentation); epistaxis and easy bruising due to thrombocytopenia. Recurrent infections (chest and urinary tract infection) due to deficiency of normal Igs. Weight loss. Low-grade fever, night sweats. Symptoms caused by hypercalcemia (found in 20% of patients at diagnosis) and renal impairment (50%) - thirst, abdominal pain, nausea, and vomiting. Symptoms caused by hyperviscosity, particularly with IgA syndromes - blurred vision, headache, confusion, breathlessness, and chest pain. Symptoms of nerve root compression (paresthesiae, focal loss of function, pain (e.g. carpal tunnel syndrome), paralysis secondary to cord compression by extradural plasma cell mass (presentation in 5% of cases)). Pain and numbness in the fingers and toes in cold weather - can be caused by cryoglobulinemia. Anorexia.

Signs

Bone signs: Bone tenderness (secondary to lytic lesions, hypercalcemia, and pathologic fractures); swellings on ribs, vertebrae, and other bones; pathologic fractures. Skeletal survey may identify areas of impending fracture in weight-bearing bones prior to symptoms. Signs due to marrow infiltration - pallor (in anemia), bruising, and epistaxis (in thrombocytopenia). Evidence of infections due to immunosuppression and leucopenia. Confusion and dehydration, secondary to hypercalcemia. Signs consistent with renal failure are found in 25% at presentation; etiology can be multifactorial: interstitial nephritis, hypercalcemia and hypercalciuria, prerenal azotemia, light chain deposition disease. Motor weakness secondary to spinal cord compression. Signs consistent with amyloidosis are present in 10-15% of patients at diagnosis

Associated disorders

Amyloidosis presents in 10-15% of myeloma patients at diagnosis. Occurs more often in patients whose plasma cells produce only light chains Symptoms may include low blood pressure, and kidney, heart, and liver failure. Carpal tunnel syndrome, nephrotic syndrome, cardiac failure, and neuropathy can occur secondary to amyloid depositions. In about 5% of patients at diagnosis, myeloma is static and may not progress for months or years. They have 'smoldering myeloma' (lower tumor burden) and are not troubled by the anemia, bone disease, renal failure, and frequent infections. They are not treated with chemotherapy until disease progression, onset of symptoms, or development of new lytic bone lesions

Differential diagnosis

Metastatic carcinoma

Metastatic cancer can produce lytic lesions and plasmacytosis and may have an associated, unrelated monoclonal gammopathy.

Features: Small M component. <10% of plasma cells in bone marrow. Bone pain is less related to movement, and usually more severe at night. Signs and symptoms relating to primary cancer

Lymphoma

Lymphoma is a solid tumor of the lymphoreticular system and is subdivided into Hodgkin's and non-Hodgkin's disease by cell type. Non-Hodgkin's lymphoma is one of the most common neoplasms encountered these days. Hodgkin's lymphoma has roughly the same incidence as multiple myeloma. Usually intermediate- to high-grade.

Features: Commonly presents with lymphadenopathy alone. Fever and night sweats. Weight loss. Recurrent infection due to immunosuppression. Pruritus. Hepatosplenomegaly may be present. Median age: 50 years for non-Hodgkin's lymphoma, increasing with age. Hodgkin's disease peaks at 15-34 years and again at > 50 years. When associated with HIV, brain lymphoma predominates

Primary neoplasm of bone and cartilage

Invasive, anaplastic tumors that can metastasize. Examples include osteosarcoma, chondrosarcoma, and Ewing's sarcoma.

Features: Pain and swelling in an otherwise well patient. Osteosarcoma usually arises at long bone metaphysis, usually 10-20 years; 50% are around the knee. Chondrosarcoma usually involves the pelvis, upper femur, or shoulder; usually >40 years. Ewing's sarcoma causes painful soft tissue mass and usually affects midshaft of long bone; peak age 10-15 years.

Monoclonal gammopathy of undetermined significance (MGUS)

Occurs in approx. 0.2% of patients aged 25-49 years, 2% of those aged 50-79 years, and 10% of those aged 80-90 years. It is a common condition where a monoclonal protein is present, other criteria for myeloma diagnosis are absent, and no cause can be identified. One study reported that 16% of cases of MGUS develop into multiple myeloma during long-term follow-up of 30 years or more. No laboratory tests are currently available that can predict which patients with MGUS will progress to multiple myeloma.

Features: It presents in asymptomatic patients with: M component of< 3g/dL. <10% bone marrow plasma cells. Absence of osteolytic lesions, anemia, hypercalcemia, or renal insufficiency. No or only small amounts of M protein in the urine (Bence Jones protein). The serum and urinary M protein should be periodically measured, and clinical and other laboratory features should be re-evaluated, to determine whether other lymphoproliferative disorders have developed.

Plasmacytoma

Rare solid tumors, which may predispose to myeloma.

Features:

Solitary plasmacytoma: Usually located in the spine or long bones of the extremities. Diagnosis is based on histologic evidence of a plasma cell tumor. Complete skeletal radiographs must show no other lesions. Bone marrow aspirate must contain no evidence of multiple myeloma. No M protein on urine or serum. Overt multiple myeloma develops in approx. 55% of patients. New bone lesions or local recurrence develop in about 10%. Progression usually occurs within 3-4 years.

Extramedullary plasmacytoma:

Plasma cell tumor that arises outside the bone marrow. In 80% of cases it is located in the upper respiratory tract (nasal cavity, sinuses, nasopharynx, and larynx). May also occur in the gastrointestinal tract, central nervous system, urinary bladder, thyroid, breast, testes, parotid gland, and lymph nodes. There is a predominance of immunoglobulin (Ig)A M protein. Diagnosis is based on the finding of a plasma cell tumor in an extramedullary location and the absence of multiple myeloma on bone marrow examination, radiography, and appropriate studies of serum and urine. Treatment consists of radiotherapy. Prognosis is favorable. Regional recurrences develop in approx. 25% of patients. Development of typical multiple myeloma is uncommon

Idiopathic Bence-Jones proteinuria

Small traces of Bence Jones proteinuria are common. Patients excrete only small amounts of this protein (60mg/L) - only 2-3% of these patients excrete greater quantities. In most patients who excrete >1g Bence Jones protein in 24h without evidence of malignant plasma cell proliferation, multiple myeloma or amyloidosis will eventually develop (but it may not occur for up to 20 years).

Features: Bence Jones protein is excreted in urine by approx.:
70% of myeloma patients. 30% of patients with Waldenstrom's macroglobulinemia. 20% of patients with lymphoproliferative malignancy. 10% of patients with so-called benign (secondary and idiopathic) monoclonal gammopathy. If Bence-Jones proteinuria is the only abnormality, this is not indicative of malignancy but careful follow-up is needed.

Sarcoidosis

Sarcoidosis is a chronic systemic granulomatous disease of unknown cause. Nonspecific caseating granulomas are characteristic.

Features: Fatigue, weight loss, anorexia, malaise. Hypercalcemia leading to bone pain, renal stones. Hypergammaglobulinemia (usually nonmonoclonal). Blurred vision, ocular discomfort, uveitis. Skin papules and macules, erythema nodosum. Arrhythmias, cardiomyopathy. Hepatosplenomegaly. Cranial nerve palsies. Pulmonary manifestations - dry cough, dyspnea. diabetes insipidus. parotid enlargement

Tuberculosis

Tuberculosis is an infection caused by the bacterium *Mycobacterium tuberculosis*, causing systemic symptoms which may resemble those of myeloma. Extrapulmonary tuberculosis may affect bone and, therefore, may have further features in common with myeloma.

Features: Weight loss. Night sweats. Fever. Cough and dyspnea. Large joint arthritis with effusion. Spondylitis of spine, often lower thoracic. Parasinous abscess. Cord compression

Aplastic anemia

A primary bone marrow failure characterized by stem cell destruction or suppression. Aplastic anemia is usually immunologically mediated. Etiology is unknown in > 50% of patients but common etiologic factors include toxins (e.g. benzene, insecticides), drugs, ionizing irradiation, and infections (e.g. HIV). It may be inherited (Fanconi's anemia).

Features: Anemia. Thrombocytopenia. Immunosuppression. Pancytopenia on complete blood count (CBC). Reticulocytopenia. No monoclonal bands or Bence Jones proteins

Myelodysplastic syndromes

A group of acquired clonal disorders of hematopoiesis affecting stem cells. It is associated with abnormal hematopoeitic maturation, and bone marrow hypercellularity but peripheral blood cytopenia. 50-90% of cases are associated with chromosomal abnormalities.

Features: Fever. Fatigue. Recurrent infection. Dyspnea. Bruising. Anemia. Patients most commonly > 65 years

Paget's disease of bone

Paget's disease is a nonmetabolic disease of bone. Osteolytic foci trigger excessive attempts at repair, resulting in heavy but weakened and distorted areas of the bone. It may be mono-ostotic or polyostotic; long bones, pelvis, and skull are commonly involved. 5-10% of patients develop bone sarcomas (osteosarcoma, malignant fibrous histiocytoma, or chondrosarcoma).

Features: Bone pain. Bowing of long bones. Pathologic fractures. Headache. Cranial nerve compression at exit foramina. Cord compression due to vertebral involvement. Increased heat over lesions. Kyphoscoliosis. Secondary osteoarthritis. High output cardiac failure. Raised serum alkaline phosphatase but normocalcemia

Human Immunodeficiency Virus (HIV)

HIV is an RNA retroviral infection leading to depletion of T helper cells and severe immunodeficiency. Usually intermediate- to high-grade.

Features: Immunosuppression characterized by falling CD4 count. Weight loss. Night sweats. Diarrhea. May present with generalized lymphadenopathy/ sore throat, headache, fever, and rash/ recurrent infections typical of T cell immunodeficiency/ lymphoma and other unusual tumors

Summary of tests

- Complete blood count (CBC) to look for anemia, thrombocytopenia, or neutropenia. Useful in all tired patients or patients losing weight
- Erythrocyte sedimentation rate (ESR) is raised if there is significant monoclonal Ig secretion, very high in hyperviscosity. Usually very high in myeloma but nonspecific
- Blood urea nitrogen (BUN) and creatinine should be used to assess renal function
- Serum uric acid is raised in myeloma but is nonspecific
- Serum total protein is raised in myeloma
- Serum calcium level is required to detect hypercalcemia
- Serum alkaline phosphatase suggests pathologic fractures or alternative diagnosis when raised
- Serum LDH
- 24-h excretion of protein and protein electrophoresis (PEP) and immunoelectrophoresis (IEP) of a concentrated specimen, and urine Bence Jones protein
- PEP, IEP, and quantitative Igs (QIG) - serum plasma protein immunoelectrophoresis for monoclonal bands is positive in >75% of patients, who also show decreased levels of normal Igs
- Plasma viscosity is used to detect hyperviscosity, but viscosity levels do not correlate well with clinical signs and symptoms. Treatment with plasmapheresis is indicated in symptomatic patients regardless of plasma viscosity levels
- Serum beta2-microglobulin measures disease activity and is a guide to prognosis. Beta2-microglobulin is a protein that is shed by B cells and correlates with myeloma cell mass. Levels >2.5mg/L are associated with a poorer prognosis
- Bone marrow aspirate and biopsy may demonstrate proliferating plasma cell line and is the most likely test to give definitive diagnosis. This is normally performed by a specialist. Samples are usually obtained from iliac crest, and then the number of plasma cells present is counted for diagnosis and staging. Plasma cell labeling index (PCLI) indicates the percentage of plasma cells that are actively dividing
- Complete skeletal survey by X-ray, including skull and long bones X-rays, is an essential part of staging and will show lytic lesions
- Technetium-99 bone scan is said to be of no benefit as lesions are lytic. Positive bone scans in myeloma usually indicate regions of fracture or arthritis - it should not be ordered as part of staging
- Order MRI of spinal cord if there is a paraspinal mass or signs of cord or nerve root compression

Tests

Complete blood count (CBC)

Cause of abnormal result
In myeloma, the red cell count and the platelet count are low, the white cell count is frequently normal until marrow replacement is extensive. Lower values than these in all cell lines suggest marrow replacement or failure. Normocytic normochromic anemia is usual in myeloma and is caused by replacement of the normal marrow by tumor cells and inhibition of hemopoiesis 67% of myeloma patients are anemic at presentation. Medications, disorders and other factors that may alter results. Drugs that may suppress bone marrow include: Chemotherapeutic agents, e.g. cyclophosphamide, Quinine, Digitalis, NSAIDs, Sulfa, penicillin, cephalosporins, Phenytoin. Other neoplasms, Myelodysplastic syndromes

Erythrocyte sedimentation rate (ESR)

Abnormal: Above the normal range. Hyperviscosity syndromes will tend to lower ESR. Myeloma accounts for 5% of levels >100. Raised in 90% of cases of myeloma at presentation

Cause of abnormal result: ESR is raised in multiple myeloma due to an increase in globulins and the presence of paraproteins. Fibrinogen is increased in malignancy, which raises ESR. Anemia can raise the ESR

Medications, disorders and other factors that may alter results

Steroids may lower ESR level.

Blood urea nitrogen (BUN) and creatinine

Cause of abnormal result: Renal failure (interstitial nephritis, hypercalcemia and hypercalciuria, prerenal azotemia, light chain deposition disease). Renal outflow obstruction. Amyloidosis

Medications, disorders and other factors that may alter results

Urea nitrogen raised by: drugs e.g. aminoglycosides, diuretics, lithium, corticosteroids, anabolic steroid abuse. Creatinine raised by: antibiotics (aminoglycosides, cephalosporins, hydantoin, diuretics, methyldopa)

Serum uric acid

Abnormal: Raised in myeloma. Keep in mind the possibility of a false-positive result

Cause of abnormal result: Raised in: Renal failure, Myeloproliferative disorders, Cell lysis, e.g. With chemotherapy, radiotherapy.

Medications, disorders and other factors that may alter results

High-protein or high-purine diet, Diuretics, Ethambutol, Nicotinic acid, Gout, Addison's disease, Active psoriasis, Acidosis

Serum total protein

Abnormal: Levels raised in myeloma. Keep in mind the possibility of a false-positive result

Cause of abnormal result: Raised in: Dehydration, Myeloma, Waldenstrom's macroglobulinemia, Sarcoidosis,Collagen vascular dis eases, MGUS

Medications, disorders and other factors that may alter results

Low-protein diet and malabsorption may falsely lower level.

Serum calcium level

If protein levels are abnormal, calcium levels need to be corrected for this. Local laboratory calibrations vary - local laboratory will advise but corrected calcium is higher if protein levels are lower.

Abnormal: Raised in myeloma due to increased osteoclast activity. Keep in mind the possibility of a false-positive result

Cause of abnormal result: Bony lysis in multiple myeloma results in substantial mobilization of calcium from bone.

Medications, disorders and other factors that may alter results

Thiazides, Lithium, Toxic levels of theophylline. Spurious due to tight cuff during phlebotomy. Hypercalcemia

Serum alkaline phosphatase

Abnormal: May be raised in myeloma if fractures are present. Normal in the absence of fractures or bone repair. Keep in mind the possibility of a false-positive result

Cause of abnormal result:Raised in: Biliary disease, Liver disease, Paget's disease of bone, Rickets, Thyroid disease, Ulcerative colitis, Bony metastases, Bone neoplasms, Cytomegalovirus and mononucleosis, Heart failure, Hypernephroma, Myelofibrosis, Leukemia, Myeloma if fractures or bone repair present

Medications, disorders and other factors that may alter results

Raised by: Estrogens, Albumin, Erythromycin, Phenothiazines, Pregnancy

Urinary Bence-Jones proteins

Advantages: Positive in about 70% of cases for light chains. One of diagnostic criteria for myeloma if positive. Negative urine immunoelectrophoresis and negative serum immunoelectrophoresis exclude myeloma in 99% of cases

Disadvantages: Negative result does not exclude myeloma. Positive result does not confirm myeloma alone. Inconsistent results frequently hinder diagnosis

Normal: Normal result is negative but small amounts of monoclonal light chains (Bence Jones proteinuria) are not uncommon.

Abnormal: Positive - when myeloma produces light chain immunoglobulin only (22% of cases), it is small enough to cross renal basement membrane and be excreted in the urine. Keep in mind the possibility of a false-positive result

Cause of abnormal result: Myeloma

Idiopathic Bence Jones proteinuria - Bence Jones proteinuria may be 'benign'. In most patients who excrete 1g of Bence Jones protein in 24 h without evidence of malignant plasma cell proliferation, multiple myeloma will eventually develop. This change may not occur for up to 20 years.Serum protein immunoelectrophoresis

Normal: Serum proteins immunoelectrophoresis: Total protein - 6.00-8.30g/dL Alpha-2-0.38-0.84g/dL. Albumin - 3.75-5.01g/dL.Beta - 0.60-0.99g/dL. Alpha-1 - 0.26-0.56g/dL. Gamma - 0.72-1.46g/dL

Abnormal: Paraprotein bands (M proteins. M spike in approx. 80% of patients with myeloma. Keep in mind the possibility of a false-positive result

Cause of abnormal result: The malignant plasma cells produce one specific protein, which is always exactly the same in one patient. M proteins show up as a 'spike': 50% are IgG protein, 20% IgA, 17% free monoclonal light chains. Levels of functional Ig are depressed in individuals with multiple myeloma. It appears that the functional Ig made by existing normal plasma cells breaks down more quickly in patients with multiple myeloma than in healthy individuals.Paraprotein bands may suggest myeloma but are also sometimes seen in: connective tissue disease, plasmacytoma, lymphoma, amyloidosis, chronic lymphatic leukemia, MGUS, Waldenstrom's macroglobulinemia, some solid tumors

Plasma viscosity

Description: Can be used to detect hyperviscosity, but viscosity levels do not correlate well with clinical signs and symptoms. Treatment with plasmapheresis is indicated in symptomatic patients regardless of plasma viscosity levels However, the plasma viscosity level is useful for the detection of asymptomatic hyperviscosity, which requires treatment in certain clinical settings (e.g. nonambulatory patients, or patients with additional risk factors for the development of venous thrombosis)

Normal: 1.4-1.8 relative to water.

Abnormal: Raised. Symptoms likely to occur at levels >4.0 (although levels do not correlate well with symptoms). Keep in mind the possibility of a false-positive result

Cause of abnormal result: Raised in: Monoclonal gammopathies - multiple myeloma, Waldenstrom's macroglobulinemia, monoclonal gammopathy of unknown significance (MGUS); the incidence is highest in Waldenstrom's macroglobulinemia with IgM, followed by IgA myeloma, and the syndrome is observed usually when serum viscosity exceeds 4.0 centipoule (cp) units relative to normal serum. Hyperfibrinogenemia

X-ray

Abnormal: Typical X-ray lesions: rounded punched out lytic lesions corresponding with pain site; in the ribs - osteolytic lesions with the appearance of diffuse mottling; in the spine - rarefaction, globular tumor formation, shortening and twisting of the vertebral column, and disappearance of intervertebral disks. Skull often shows punched out lesions with no sclerotic or reactive border; these may be multiple, the so-called 'pepperpot skull'. Periosteal reaction is uncommon. Vertebral compression fractures are often seen. 0.5-3.0% of the patients have mainly osteosclerotic lesions: sites -thoracic and lumbar spine, the tibia and fibula, the scapula, and sites of tendon and ligament insertions of the hands; X-ray appearance - irregular, fluffy, or spiculated; must be differentiated from metastases of breast or prostate carcinomas. Occasionally, a mixture of lytic and sclerotic lesions is seen

Cause of abnormal result: Myeloma. Secondary tumor deposit from another primary. Prostate and breast secondaries usually cause sclerotic lesions but most others are lytic .Osteoporosis. Normal impact fracture: compare with history. Fracture with minimal or no force is usually pathologic.

Clinical Hallmarks

Serum interleukin (IL)-6 levels correlate with disease activity and tumor cell mass. Increased IL-6 serum levels in almost all patients in terminal phase shows that this cytokine is an important factor in the progression the disease Early in the disease, Streptococcus pneumoniae is the most common pathogen, but infections with Haemophilus influenzae or other streptococci are also seen. With disease progression and therapy, infections caused by Staphylococcus aureus predominate, accounting for 80% of all septic complications and most deaths related to infection.

INTERPRETATION

DIAGNOSING AND STAGING MULTIPLE MYELOMA, INTERPRETING INVESTIGATION RESULTS.

Diagnostic criteria for multiple myeloma according to Durie and Salmon diagnostic criteria require the presence of the following criteria:

- Any two major criteria
- Major criterion 1 plus minor criterion b, c, or d
- Major criterion 3 plus minor criterion a or c
- Minor criteria a, b, and c, or a, b, and d

Major criteria:

1. Plasmacytoma on tissue biopsy
2. Bone marrow plasmacytosis with >30% of plasma cells
3. M protein - IgG >3.5g/L; IgA >2.0g/L, kappa or lambda chain excretion on urine electrophoresis >1g/24h in the absence of amyloidosis

Minor criteria:

a. Bone marrow plasmacytosis with 10-30% plasma cells
b. Detection of an M protein in serum or urine but less than levels defined above
c. Lytic bone lesion
d. Residual normal IgM < 500mg/L, IgA <1g/L, or IgG < 6g/L

Diagnostic decision for indolent multiple myeloma:

- Criteria as for myeloma with the following limitation
- Absent or only limited bone lesions (= 3 lytic lesions), no compression fracture
- Paraprotein levels IgG < 7.0g/dL, IgA < 5.0g/dL
- No symptoms or associated disease features - hemoglobin >10mg/dL, serum creatinine < 2mg/dL, normal serum calcium, and no infections

Diagnostic decision for smoldering myeloma:

- As for indolent multiple myeloma with additional constraints
- Bone marrow plasma cells 10-30%
- No bone lesions

The recognition of this subset of patients is crucial because they should not be treated unless progression occurs

MGUS:

- Paraprotein levels IgG =3.5g/dL; IgA =2.0g/dL; Bence Jones protein =1.0g/24h, Bone marrow plasma cells <10%, No bone lesions, symptoms

Solitary plasmacytoma:

- Single plasma cell tumor, No diagnostic criteria for systemic myeloma, Little or no paraprotein after local therapy, <10% plasma cells in the bone marrow

Clinical and laboratory evaluation of patients with monoclonal gammopathies:

- Serum and urine electrophoresis of high resolution is indicated for all patients suspected of having a plasma cell dyscrasia. The quantitative level of M protein should be defined precisely by densitometry
- Quantitation of 24h urine protein excretion to assess the presence, type, and daily excretion of monoclonal free light chains
- High-resolution electrophoresis assesses changes in level of a previously identified monoclonal protein in serum or urine at regular intervals that vary from every 1-2 months for patients being treated for multiple myeloma
- Hyperviscosity syndrome requires emergency plasma exchange with indications based on clinical features. Serum viscosity and serum protein electrophoresis are recommended prior to the first plasma exchange to correlate the level of M protein with symptoms in that patient. This correlation may be used to anticipate repeat plasma exchanges as the M protein approaches the level associated with hyperviscosity

Staging of multiple myeloma:

- Classification of Durie and Salmon is used for staging multiple myeloma
- This system correlates well with tumor mass and prognosis
- Stage is determined by level of M protein, number of lytic bone lesions, hemoglobin concentration, and serum calcium level
- Patients are further subdivided into classes A and B on the basis of the serum creatinine level

Stage I

All of the following must be present:

- Hemoglobin >10g/dL, Serum calcium: less or equal to 12mg/dL, Normal bone structure or solitary plasmacytoma on radiographs, Low M component, IgG < 5g/dL, IgA < 3g/dL,Urine light chains < 4g/24h

Stage II

- Not fitting stage I or III

Stage III

One or more of the following:

- Hemoglobin < 8.5g/dL, Serum calcium >12mg/dL, Advanced lytic bone lesions - more than three sites of bone damage, Hyper M component, IgG > 7g/dL, IgA > 5g/dL, Urinary light chain excretion > 12g/24h

BOUQUET

IN LIGHTER VEIN

Neurotics build castles in the sky.
Psychotics live in them.
Psychiatrists collect the rent.

Welcome to the Psychiatric Hotline.
If you are obsessive-compulsive, please press 1 repeatedly.
If you are co-dependent, please ask someone to press 2.
If you have multiple personalities, please press 3, 4, 5, and 6.
If you are paranoid-delusional, we know who you are and what you want. Just stay on the line so we can trace the call.
If you are schizophrenic, listen carefully and a little voice will tell you which number to press.
If you are depressed, it doesn't matter which number you press. No one will answer.
If you are delusional and occasionally hallucinate, please be aware that the thing you are holding on the side of your head is alive and about to bite off your ear.

WISDOM WHISPERS

- Anyone who has never made a mistake has never tried anything new.
- You may be disappointed if you fail, but you are doomed if you don't try.
- Mistakes are the portals of discovery.
- If you want a place in the sun, you've got to put up with a few blisters.
- Compromise: An amiable arrangement between husband and wife whereby they agree to let her have her own way.

BRAIN TEASERS

1. Hypersegmented neutrophilic nuclei are seen in which of the following conditions?
   A. Megaloblastic anemia B. Hypochromic anemia C. Hemolytic anemia D.PNH
2. In which of the following poisonings is RBC basophilic stippling observed?
   A. Copper B. Lead C. Silver D. Arsenic

Answers: 1. A, 2. B,

TROUBLE SHOOTING

GUIDELINES FOR BLOOD GROUPING AND ANTIBODY TESTING IN PREGNANCY

(....continued)

Antibody screening

Approximately 1% of pregnant women are found to have clinically significant red cell antibodies. Of these, the commonest specificity is anti-D, although the universal introduction of RAADP is predicted to reduce this sensitisation rate. However, with the introduction of RAADP there is a significant rise in positive antibody screening results, due to the detection of prophylactic anti-D.

Screening methods

The Indirect Antiglobulin Test [IAT] using reagent red cells suspended in LISS is the most suitable for detection of clinically significant red cell antibodies. Column agglutination methods, liquid-phase tube tests and solid-phase methods have been found to be suitable. There is no additional value in using an enzyme technique in antibody screening. Testing for high levels of immune anti-A or anti-B in pregnant women is not recommended as their presence neither predicts ABO HDN nor does it cause problems in utero.

Reagent cells

Cells used for antibody screening should comply with the recommendations of the guidelines for compatibility procedures in blood transfusion laboratories

The following antigens should be expressed on screening cells:

C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b,S, s, M, N, Le^a.

It is recommended that one of the screening cells should be R_1R_1 and another should be R_2R_2 and that the Fy^a, Fy^b, Jk^a, Jk^b, S and s antigens should be represented on reagent cells with homozygous expression. Screening cells must not be pooled.

It is not necessary to include screening cells which express low frequency antigens such as C^w, Kp^a or Lu^a.

Recommendation 5:

The screening cells and methods used for red cell antibody screening should comply with the guidelines for compatibility procedures in blood transfusion laboratories.

ANTENATAL TESTING PROTOCOLS

See algorithm for samples and testing requirements.

Routine Testing

All pregnant women should have samples taken early in pregnancy, ideally at 10-16 weeks gestation, for ABO and D typing and for screening for the presence of red cell alloantibodies. When an antibody screen is positive further tests should be carried out to determine the antibody specificity and significance.

All pregnant women, whether D positive or D negative, should have a further blood sample taken at 28 weeks gestation for re-checking the ABO and D group and further screening for red cell allo-antibodies. D positive women are just as likely as D negative women to form antibodies, other than anti-D, late in pregnancy. No further routine blood grouping or antibody screening is necessary after 28 weeks.

There is evidence that antibodies detected only in the third trimester do not cause HDN. Further, and significantly, the introduction of RAADP has resulted in the detection of anti-D in samples taken after 28 weeks from D negative women. Since it is not possible to differentiate between prophylactic and immune anti-D there is the potential for confusion between the two.

Recommendation 6:

All pregnant women should be ABO and D typed and screened for the presence of red cell antibodies early in pregnancy and at 28 weeks gestation

Local policies must ensure that D-negative women eligible for RAADP have the third trimester antibody screening sample taken before the first RAADP injection is administered at 28 weeks. Samples taken after the injection could result in passive anti-D being detected which may be mistaken for immune anti-D.

Sensitising Episodes during pregnancy

This section is a synopsis of recommendations of the Guideline for administration of anti-D prophylaxis.

In addition to RAADP, pregnant women who are D negative should be considered for anti-D prophylaxis for the following potentially sensitising episodes:

- Amniocentesis
- Cordocentesis
- Other in-utero therapeutic intervention/surgery (e.g. Intrauterine transfusion, shunting)
- Ante partum haemorrhage (APH)
- Chorionic villus sampling
- Ectopic pregnancy
- External cephalic version
- Fall / Abdominal trauma
- Intrauterine death
- Miscarriage
- Termination of pregnancy

Before 12 weeks gestation, confirmed by scan, in uncomplicated miscarriage or mild and painless vaginal bleeding, anti-D should not be administered because the risk of feto-maternal haemorrhage [FMH] is minimal.

Between 12 and 20 weeks gestation, for any potential sensitising event outlined above, a sample should be tested to confirm that the woman is D negative and that she has not become sensitised to the D antigen. At least 250 IU anti-D immunoglobulin should be administered as soon as possible and certainly within 72 hours of the event. There is no need to assess the volume of FMH under 20 weeks gestation.

After 20 weeks gestation there is a requirement to assess the volume of FMH.

If the acid elution technique is used and a FMH of more than 4mL is indicated, the test should be repeated using flow cytometry. At least 500 IU anti-D immunoglobulin should be administered intramuscularly as soon as possible, and certainly within 72 hours of the potentially sensitising event. If FMH of more than 4mL is confirmed by flow cytometry, more anti-D will be required.

After the birth, a cord sample must be tested to obtain the ABO and D type of the baby. If this is not collected for any reason, a heel prick sample should be obtained as soon as possible.

Maternal samples for confirmatory ABO and D type and FMH testing should be collected after sufficient time has elapsed for any FMH to be dispersed in the maternal circulation. A period of 30-45 minutes is considered adequate.

Following birth of a D positive infant at least 500 IU anti-D must be administered to the woman if the FMH is 4 mL or less.

If the pregnancy is non-viable and no sample can be obtained from the baby, prophylactic anti-D must be administered to the woman.

Red cell antibodies detected in pregnancy:

When red cell antibodies are detected, further testing of maternal blood should be undertaken to determine the specificity, concentration, origin and level of antibody or antibodies, and the likelihood of HDN.

Anti-D, anti-c and anti-K are the antibodies most often implicated in causing haemolytic disease severe enough to warrant antenatal intervention.

Women with anti-D present

Distinguishing between prophylactic and immune anti-D

In addition to the administration of prophylactic anti-D following sensitising events the use of RAADP is increasing. It is therefore inevitable that more antenatal samples containing low levels of anti-D will present the problem of determining whether the anti-D is prophylactic or immune.

Following administration of an intramuscular injection of anti-D immunoglobulin, serologically detectable levels of anti-D are quickly reached and peak blood levels are reached within three to seven days. The half-life of prophylactic anti- D immunoglobulin is approximately 3 weeks. Prophylactic anti-D can be detected by serological tests for several weeks: by IAT for up to 8 weeks or more and by other more sensitive techniques for up to 12 weeks and in exceptional cases for several months.

Immune anti-D becomes detectable approximately 4 weeks after exposure to D positive cells, and reaches a peak level after 6-8 weeks.

Both prophylactic and immune anti-D are detectable by laboratory tests and cannot be distinguished. While prophylactic anti-D levels will fall with time, immune anti-D levels will usually remain stable or rise if there is re-stimulation of the antibody.

The level of anti-D in maternal samples post prophylaxis rarely exceeds 1 IU/mL unless a dose[s] of more than1250 IU has been administered.

Procedure if prophylactic anti-D is suspected

a. If there is a record of administration of anti-D within the past 8 weeks and the antibody reaction is weak, testing should be as for non-sensitised women i.e. no antibody testing after 28 weeks and Rh prophylaxis should continue.
b. If there is no record of anti-D administration or information regarding prophylaxis is not available the antibody should be monitored by both IAT and anti-D quantification as for immunised women i.e. at 4 weekly intervals to 28 weeks or at 2 weekly intervals if after 28 weeks. If the anti-D becomes undetectable by IAT and the quantified level is falling it is probably prophylactic. A rising or steady level indicates immune anti-D.

If there is significant doubt about the immune or passive nature of anti D, the sample should be referred for quantification.

In either case anti-D prophylaxis should continue unless it is established beyond doubt that the anti-D is immune.

The pregnant woman with anti-D should not be issued with an antibody card documenting the finding of anti-D until it is established that the anti-D is immune.

If the sample in which anti-D is detected is referred for routine antibody screening or is pre-transfusion, a panel of D negative cells, selected to provide all relevant red cell antigens, should be used to detect or exclude the presence of allo-antibodies of other specificities.

Recommendation 7: Blood transfusion laboratories should keep a record of anti-D administration to provide a basis for distinguishing between immune and prophylactic anti-D.

Women with immune anti-D

Blood samples from women with immune anti-D should normally be tested at least monthly until 28 weeks gestation and every 2 weeks thereafter to monitor the level of anti-D and to identify any additional antibodies that may develop.

The antibody level should be quantified, in IU/mL, using the established anti-D standard.

Each sample should be tested in parallel with the previous sample and the results compared to identify significant changes in antibody level.

Where the level is more than 1.0 IU/mL an increase of anti-D level of 50% or greater over the previous level indicates a significant increase, irrespective of the period of gestation.

Anti-D is the most frequent antibody responsible for serious HDN. The following levels of anti-D have been used to guide the management of pregnancies since the publication of the previous guideline.

- Anti-D Less than 4 IU/mL HDN unlikely
- Anti-D 4-15 IU/mL Moderate risk of HDN
- Anti-D More than 15 IU/mL High risk of hydrops fetalis

As a consequence of developments in the assessment of fetal anaemia and in the technique of IUT the significant anti-D level is that which triggers referral to a specialist feto-maternal unit. Non-invasive assessment can then be used to monitor fetal anaemia . A woman whose anti-D level is 4 IU/mL or greater and/or has a rising anti-D level and/or has a history of HDN affected offspring must be referred to such a unit. It should also be noted that HDN has been reported at levels less than 4 iu/mL. Once the referral to the feto-maternal unit has been made the value of subsequent samples for anti-D quantification is doubtful. A sample at 28 weeks should be tested for the presence of further red cell antibodies.

It is possible to determine the D type of the fetus from a maternal peripheral blood sample using polymerase chain reaction (PCR).

Women with apparent anti-C + D, possible anti-G

A proportion of antibodies with apparent anti-C+D specificity but with disproportionately high anti-C titres may be demonstrated, by advanced serological techniques, to be anti-G. Since women with anti-G, without anti-D, should be eligible for RAADP and post-delivery anti-D immunoglobulin it is important that a reference centre should confirm examples of apparent anti-C+D specificity.

Women with immune anti-c present

Women with anti-c should be re-tested with the same frequency as women with anti-D, i.e. at least monthly to 28 weeks gestation and every 2 weeks thereafter.

Samples from women with anti-c should be quantified with reference to the international anti-c standard with the previous sample tested in parallel, as for anti-D [above], and any additional antibodies should be identified.

Quantification of anti-c is useful in monitoring increases in the antibody concentration.

When account has been taken of previous history of HDN the following levels of anti-c are indicative of the need to refer to a specialist unit

Anti-c level:

- Less than 7.5 IU/mL Continue to monitor.
- 7.5 to 20 IU/mL Risk of moderate HDN, refer to specialist unit.
- More than 20 IU/mL Risk of severe HDN Refer to specialist unit.

It is important to note that anti-c may cause delayed anaemia in the neonate.

(to be continued...)