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  
**History:** No previous history of transfusion, patient having cardiac problem.

### Other Information

Tulip is known for developing excellent Anti-A1 lectin for A1 group. In this case we identified a rare A3 group. Tulip Diagnostics is the only company to develop Anti-A1 lectin reagents.

### New Test Case

The patient’s blood sample could be a weak A Rh+ve group. Therefore the sample could be of A3 group. Further one more evaluation test for confirming A3 group was conducted using the serum of a known O group and B group person as mentioned in Blood Transfusion in Clinical Medicine by P.L. Mollison, 10th Edition.

#### Observations:

1. **Forward grouping ABD (Tube test):**
   - Patient’s blood sample showed weak reaction (1+) with Anti-A as expected in known A2 group samples (no reaction).
   - A2 group and known A2 group samples gave no reaction with Anti-H lectin reagents.

2. **Reverse grouping of the serum sample with known cells:**
   - Both patient’s serum sample & known A1 group serum showed no reaction with A1 Rh+ve cells and presented a 3+ reaction. Therefore patient’s sample is not A1 (stronger subgroup of A).

3. **Tube test with Erybank Anti-H lectin:**
   - Patient’s blood sample was found to react negatively with known A1 and A2 group serum samples using Eryscreen Anti-A antibody.

4. **Tube test with Erybank Anti-A1 lectin:**
   - Observation:
     - Patient’s blood sample showed no reaction with Anti-A1 lectin as expected in known A1 group samples. Patient’s sample presented a 4+ reaction. Therefore patient’s sample is an A1 group sample. A1 group is a weaker subgroup of A group (i.e., A2, A3, Ax etc).

5. **Test patient cells with known O and B group serum:**
   - Both patient’s sample and known A1 group sample gave strong reaction (3+) with Anti-D (IgM). Other information about serum patient cells:

<table>
<thead>
<tr>
<th>Reagent Lot. No</th>
<th>Patient’s sample</th>
<th>Known A1 group</th>
<th>Known A2 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eryscreen Anti-A</td>
<td>124627</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>Eryscreen Anti-B</td>
<td>124627</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eryscreen Anti-D</td>
<td>124627</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>

### Troubleshooting

Further to this at Tulip we carried the following tests:

1. **Trouble shooting A:**
   - No previous history of transfusion, patient having cardiac problem.
   - A request came a couple of months back to consider in detail a not so uncommonly encountered neoplastic disease called as Plasmacytoma/Multiple Myeloma.

2. **Interpretation:**
   - As Pregnancy and related Immunohaematology could 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.

3. **Tulip News:**
   - Between the covers, every word shall ooze sense when squeezed. So just go ahead right now, right away.
DISEASE DIAGNOSIS

MULTIPLE MYELOMA

Description

Secondary neoplasm of bone and cartilage. A plasma cell tumor that arises outside the bone marrow. In 80% of cases it is associated with chronic inflammatory disease of the upper respiratory tract (nasal cavity, sinuses, nasopharynx, and oropharynx). May also occur in the gastrointestinal tract, central nervous system, and skin. Idiopathic Bence-Jones proteinuria is a renal manifestation of plasma cell dyscrasias.

Diagnosis

Clinical presentation

Multiple myeloma is diagnosed when at least three of the following four criteria are met: a) an abnormal serum protein electrophoresis, b) a bone lesion on x-ray, c) an increased bone turnover as demonstrated by increased serum alkaline phosphatase or decreased 24-hour urinary hydroxyproline, and d) a bone marrow aspirate and biopsy showing more than 30% plasma cells and greater than 5% plasma cells with the features of plasma cell dyscrasia.

Epidemiology

Incidence

Prevalence: 10 per 100,000. The apparent increase in incidence is likely related to the increased availability and use of medical facilities, and to better diagnostic procedures.

Demographics

Predominantly age 40-85 years. Peak incidence in seventh decade. Median age 69 years (men), 71 years (women). Only 15% of sufferers are <50 years.

Comorbid conditions

May complicate severity of the illness (e.g., diabetes, renal failure).

Pathogenesis

Abnormal karyotypes are present in 30-40% of patients but there is no specific cytogenetic abnormality. Chromosome abnormalities are present in about 32% of patients at the time of diagnosis. Myeloma cells are of the plasma cell type and require the continuous production of monoclonal immunoglobulin. The presence of monoclonal gammopathy is termed monoclonal gammopathy of undetermined significance (MGUS). Myeloma cells then become malignant and can cause bone pain, hypercalcemia, hyperiongulinaemia, renal impairment, and osteolytic bone lesions. Hypercalcemia is a major cause of morbidity and mortality in patients with multiple myeloma and can be caused by increased bone turnover, increased renal tubular reabsorption of calcium, and decreased renal excretion of calcium. The bone disease in multiple myeloma is characterized by bone resorption and formation, resulting in osteolysis and osteosclerosis. Osteolysis is due to the action of osteoclasts, which are activated by cytokines released by the myeloma cells. Osteosclerosis is due to the action of osteoblasts, which are activated by cytokines released by the myeloma cells. The result is a net loss of bone mass and increased bone turnover. The bone disease in multiple myeloma is characterized by bone resorption and formation, resulting in osteolysis and osteosclerosis. Osteolysis is due to the action of osteoclasts, which are activated by cytokines released by the myeloma cells. Osteosclerosis is due to the action of osteoblasts, which are activated by cytokines released by the myeloma cells. The result is a net loss of bone mass and increased bone turnover.

Clinical symptoms

Fatigue, weight loss, anorexia, malaise. Hypercalcemia leading to nausea, vomiting, constipation, and mental status changes. Osteolytic bone lesions leading to pain, weakness, and deformity. Renal impairment leading to azotemia, hypercalcemia, and hyperiongulinaemia. Hyperiongulinaemia leading to hypercalcemia, hyperiongulinaemia, and hyperviscosity. Hyperviscosity leading to neurologic symptoms, such as headaches, dizziness, and confusion. Cardiac impairment leading to angina, dyspnea, and congestive heart failure. Pulmonary impairment leading to dyspnea, cough, and hypoxia. GI tract impairment leading to nausea, vomiting, and diarrhea. GI tract impairment leading to nausea, vomiting, and diarrhea. GI tract impairment leading to nausea, vomiting, and diarrhea. GI tract impairment leading to nausea, vomiting, and diarrhea.

Differential diagnosis

Monoclonal gammopathy of undetermined significance (MGUS). Myeloma cells then become malignant and can cause bone pain, hypercalcemia, hyperiongulinaemia, renal impairment, and osteolytic bone lesions. Hypercalcemia is a major cause of morbidity and mortality in patients with multiple myeloma and can be caused by increased bone turnover, increased renal tubular reabsorption of calcium, and decreased renal excretion of calcium. The bone disease in multiple myeloma is characterized by bone resorption and formation, resulting in osteolysis and osteosclerosis. Osteolysis is due to the action of osteoclasts, which are activated by cytokines released by the myeloma cells. Osteosclerosis is due to the action of osteoblasts, which are activated by cytokines released by the myeloma cells. The result is a net loss of bone mass and increased bone turnover. The bone disease in multiple myeloma is characterized by bone resorption and formation, resulting in osteolysis and osteosclerosis. Osteolysis is due to the action of osteoclasts, which are activated by cytokines released by the myeloma cells. Osteosclerosis is due to the action of osteoblasts, which are activated by cytokines released by the myeloma cells. The result is a net loss of bone mass and increased bone turnover.

Prognosis

Survival is generally poor, with a median survival of 3-5 years from diagnosis. The prognosis is improved in patients who undergo intensive chemotherapy followed by maintenance therapy. The median survival of patients treated with intensive chemotherapy is approximately 10 years.

Signs and symptoms

Bone pain, hypercalcemia, hyperiongulinaemia, and hyperviscosity.

Treatment

Chemotherapy is the mainstay of treatment for multiple myeloma. The most commonly used chemotherapy agents are corticosteroids, cyclophosphamide, doxorubicin, vincristine, and dexamethasone. Other agents that are used in the treatment of multiple myeloma include thalidomide, lenalidomide, bortezomib, carfilzomib, and pomalidomide. Stem cell transplantation is an option for patients who are not candidates for chemotherapy. The most common side effects of chemotherapy include myelosuppression, mucositis, and neurotoxicity.

Prevention

There is currently no known way to prevent multiple myeloma. However, certain risk factors can be reduced through lifestyle modifications, such as maintaining a healthy weight, avoiding smoking, and limiting alcohol intake.

References

Erythrocyte sedimentation rate (ESR)

- Increased in myeloma, rheumatoid arthritis, inflammatory bowel disease, and infections.
- Normal in systemic lupus erythematosus.
- Decreased in anemia, hypothyroidism, and hypoproteinemia.

Interpretation of Abnormal Results:

- Increased: Myeloma, infection, inflammation, rheumatoid arthritis, systemic lupus erythematosus, anemia, hypothyroidism, hypoproteinemia.
- Decreased: Myeloma, infection, inflammation, rheumatoid arthritis, systemic lupus erythematosus, anemia, hypothyroidism, hypoproteinemia.

**INTERPRETING INVESTIGATION RESULTS.**

**DIAGNOSING AND STAGING MULTIPLE MYELOMA.**

**Causes of abnormal result:**

- Myeloma: Bone neoplasms, Cytomegalovirus and mononucleosis, Heart failure, disease of bone, Rickets, Thyroid disease, Ulcerative colitis, Bony metastases, Medications, disorders and other factors that may alter results.

- Erythrocyte sedimentation rate (ESR) negative serum immunoelectrophoresis exclude myeloma in 99% of cases.

**Criteria for myeloma:**

- Hyperglobulinemia: Serum proteins immunoelectrophoresis: Total protein = 6.00-8.30g/dL, IgG = 3.5g/dL, IgA = 2.0g/dL, light chains IgG = 1g/24h, IgA = 1g/24h, Bence Jones protein < 1g of Bence Jones protein in 24 h.

**Paraprotein bands (M proteins. M spike in approx. 80% of patients**

- Raised in: Monoclonal gammopathies - multiple myeloma, Waldenstrom's macroglobulinemia, monoclonal gammopathy of unknown significance. M proteins show up as a single band on electrophoresis. They are usually monoclonal IgG, IgA, or light chains. They may be abnormal or normal in plasma.

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**Paraprotein levels IgG =3.5g/dL; IgA  =2.0g/dL; Bence Jones protein <1g/24h, Bone marrow plasma cells <10%, No bone lesions, serum creatinine < 2mg/dL, normal serum calcium, and no infections**

**Classification of monoclonal gammopathies:**

- Indolent multiple myeloma: Patients may be asymptomatic or have only minor symptoms. They may have a single plasma cell tumor, no diagnostic criteria for systemic myeloma, and the syndrome is observed usually when serum viscosity exceeds 4.5 cP (units with relative normal serum hyperfibrinogenemia).

**Bone and soft tissue:**

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**BONE AND SOFT TISSUE.**

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For the woman if FMH is 4-6 IU. If the pregnancy is non-rh-negative no is 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 prophyactic 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 prophyactic or immune in origin.

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 fall of the prophylactic anti-D immunoglobulin is approximately 2 weeks. Prophylactic anti-D can be detected by serological tests for between 5 days to 5 weeks or for more sensitive tests 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 at 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 no inhibition of the antibody.

The level of anti-D in maternal samples post pregnancy rarely exceeds 10 IU/L unless a dose of more than 250 IU has been administered.

Prophylactic anti-D is not expected:

- There is no record of administration of anti-D with the past levels and the antibody reaction in weekly, testing should be as for non-sensitised women.

- If there is significant doubt about the immune or passive nature of anti-D, the antibody should be monitored by both IAT and or anti-D level.

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Quantification of anti-D is useful in monitoring increases in the antibody level.

The antibody level should be quantified in IU/L 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 levels.

Where the levels rise more than 1.0 IU/L 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 severe HDN. The following levels of anti-D have been used to guide the management of pregnancies since the publication of the previous guidelines:

- Less than 1.5 IU/L "Moderate risk of HDN"
- More than 15 IU/L "High risk of HDN"

As a consequence of developments in the assessment of fetal anaemia and in the technique of IAT 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 is 4.0 IU/L or greater and who has a rising anti-D level and has a history of HDN must be referred to a specialist unit. It should also be noted that HDN has been reported at levels less than 4 IU/L. Once the referral to the feto-maternal unit has been made the value of independent 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 C4A status from a maternal peripheral blood sample using polymerase chain reaction (PCR).

Serum samples from women with anti-C are referred to a specialist unit for confirmatory testing and for the following specificities.

- Anti-C level:
- 1.5 to 4.0 IU/L Risk of severe HDN, refer to specialist unit.
- S to 7.5 IU/L Risk of moderate HDN.
- No anti-C<br>Anticardiolipin antibodies:

Women with anticardiolipin antibodies should be referred to a specialist unit for confirmatory testing and for the following specificities.

- Anti-cardiolipin level:
- Less than 10 IU/L Risk of HDN<br>Anti-phospholipid antibodies:

Women with anti-phospholipid antibodies should be referred to a specialist unit for confirmatory testing and for the following specificities.

- Anti-phospholipid level:
- Less than 10 IU/L Risk of HDN