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Hematological Neoplasms: Acute Promyelocytic Leukemia

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Historical Background

Acute Promyelocytic Leukemia, or APL, was first identified as being its own entity aside from other leukemia's in 1957. Unlike other hematological neoplasms, APL has a clearly defined clinical course since its discovery. Morphologically, APL has a characteristic appearance as the bone marrow is effaced with granulated cells arrested at an immature stage. Biologically, genetic changes define the syndrome and the consequences of these changes result in homogenous leukemogenesis. It seemed to be that APL was the most malignant form of all acute leukemias. Recognition of this leukemia as a separate entity was crucial for the clinician due to the relative common occurrence of life threatening associated coaguopathy which mandated special procedures such as aggressive blood transfusions. Yet, despite prompt diagnosis, mortality was often significantly higher than other forms of leukemia. Swedish author, Hillestad, described APL as "a very rapid fatal course of only a few weeks duration" (Wang 2505). APL became the most feared diagnosis to all clinicians in the mid-twentieth century.

In the last two decades there has been a shift of pattern with APL now being recognized as the most curable form of acute leukemia. Introduction of a new target therapy has allowed for investigation into the effect certain agents have on cellular differentiation. Consequently, these findings directed clinicians to a basic understanding of the biological aspects of APL, which subsequently has led APL to becoming a model for the entire field of cancer medicine.

Epidemiology

APL comprises 5-8% of all Leukemia's of the myeloid cell line. The disease can occur at any age, though most cases are diagnosed as adults in mid-life. A study conducted by Matthew Matasar at Colombia University observed 709 patients. 334 were men and 375 were woman, a 1:1 sex distribution ratio; therefore concluding that APL is non-sex discriminate. Racial and ethnic distributions were as follows: 438 whites, 144 Hispanics, 76 Asians, and 51 blacks. A summary of their findings is illustrated in Table 1. Analysis of this data concluded that Hispanics and blacks were found to be diagnosed in an early stage of life (before the age of 50), while Asians and whites were predominantly diagnosed after the age of 50. Further investigation as to why Hispanics and blacks were more likely to receive their diagnosis at a younger age led to the conclusion that Hispanics and blacks have a heritable genetic susceptibility that contributes to this disease. However, much remains to be understood about the epidemiology of APL.

C*linical Presentation*

Symptoms (nonspecific)

Most patients complain of fatigue, and as a result, a decrease in everyday activities. A dramatic finding in a physical examination of these patients is manifestation of a hemorrhage. There may be petechiae, or small pinpoint red patches on the surface of the skin.

Laboratory Findings

Complete Blood Count/Coagulation Studies

A CBC should be evaluated for red blood cell, white blood cell, and platelet count values. Most often, patients present with anemia, leukocytosis, and thrombocytopenia. A differential should also be performed to identify the presence/absence of abnormal cells. Coagulation studies should also be performed to see if the patient presents with

disseminated intravascular coagulation(DIC). Since the severity of DIC can range from acute, to severe, to a low grade disorder, the patient must be assessed to determine which stage they are in. This is done by considering the entire clinical picture, with both a physical and laboratory diagnosis.

Morphology

A peripheral blood smear is analyzed for cellular morphology. The nucleus of the promyelocytes may or may not have an irregular shape and/or size(Figure 1). The cytoplasm is densely packed with large primary granules, staining bright pink or purple(Figure2). The cells may be so hypergranular that they obscure the nuclear cytoplasmic margin. Characteristic cells of APL contain Auer rods, or improperly fused granules(Figure 2). Cells containing bundles of Auer rods are termed faggot cells.

Cytochemical Stains

A myeloperoxidase stain can be used to identify Auer rods and faggot cells. A MPO stains primary granules of immature cells. As expected, the MPO reaction is strongly positive for promyelocytes(Figure 3). A specific esterase reaction using chloroacetate esterase can also be performed to detect specific enzymes only found in granules of immature myeloid cells. As expected, the chloroacetate stain is strongly positive (Figure 4).

Bone Marrow

APL is a malignancy of the bone marrow in which the myeloid cell line is arrested at the promyelocytic division stage and therefore the cell line is unable to differentiate and produce mature, functional cells. A bone marrow biopsy(Figure 5) and aspirate(Figure 6) are usually obtained to assess the cellularity of the bone marrow. In these patients, the bone marrow is a homogenous field of myeloid precursors,

predominantly promyelocytes and blasts. The cellularity is usually greater than eighty percent. The myeloid: erythroid ratio is markedly increased.

Genetics

Cytogenetic studies are used within the laboratory to confirm the presence of a suspected chromosomal abnormality not detected by conventional laboratory methods. FISH, or fluorescent in situ hybridization, is a technique used to detect the presence or absence of specific DNA sequences on chromosomes. Using FISH, the reciprocal translocation between chromosome 15 and 17, or t(15:17), the definitive characteristic of APL, can be detected(Figure 7). Found in 95% of patients, the retinoic acid receptor alpha gene, RARα, on the 17q12 chromosome fuses with the nuclear regulatory factor on the 15q22 chromosome, also known as the promyelocytic leukemia (PML) gene, to form a PML-RARα gene fusion product. The PML-RARα plays a critical role in the leukomogenesis of this neoplasm. The PML/RARα is able to form homodimers which in turn repress the transcriptional expression of target genes that allow for granulocytic differentiation of cells. Hence, the ultimate result of the t(15:17) as a genetic defect is crucial for its pathogenesis.

Immunophenotype (Figure 8)

Immunophenotyping use antibodies to detect specific markers on the cell surface which are associated with cell lineage and maturation. Different markers are expressed at different stages of maturation. APL is characterized by an absence of HLA-DR and CD4. Myeloid markers, CD33 and CD117, are strongly positive as is CD2 (a T-cell marker) and CD9, a marker only expressed by the APL subtype. CD45 is also used as a gating tool to selected cells of interest.

Treatment Studies

If APL is diagnosed in time, patients can be put on treatment and go into complete remission. The treatment history can be subdivided into four periods: pre-ATRA period, introduction of ATRA, use of ATO in treatment, and ATRA/ATO combination.

Pre-ATRA: Recognition of APL as a highly fatal disease and its response to Chemotherapy

In 1973, studies suggested that APL leukemic cells were sensitive to chemotherapy, specifically daunorubicin. 55 percent of patients (study of 34 patients) went into complete emission. From then on, chemotherapy, composed of anthracycline and cytosine arabinoside, was the frontline treatment of APL. The complete remission rates reached 80% in newly diagnosed patients. However, severe bleeding episodes lead to early death rate in these patients. The mean duration of remission ranged from 11-25 months and only 35-45 percent of patients were actually cured by chemotherapy.

ATRA: introduction in APL differentiation therapy

The discovery of PML/RARα in APL pathogenesis now directed researcher's attention to a possible molecular mechanism that could underlay a specific solution towards proper treatment. In 1985, ATRA, or all trans retinoic acid, a derivative of vitamin A, was found to target the fusion of the RARα with the nuclear regulator factor by dissociating the oncogenic bond; therefore allowing DNA transcription and differentiation of immature leukemic promyelocytes into mature cells. Induction of ATRA raised the complete remission rate to 90-95 percent. Even though a high complete remission rate could be achieved with ATRA alone, continuous treatment of APL with ATRA causes progressive resistance to the drug, resulting in relapse usually within three to six months. Furthermore, the administration of ATRA induces elevation of the white blood cell count, resulting in retinoic acid syndrome. These adverse effects prompted investigators to further optimize their treatment protocol.

Use of ATO: Arsenic Trioxide

Arsenic is a naturally occurring substance that exists in organic and inorganic forms. Although arsenic is known as a poison, it has been used for centuries as a treatment drug in Western and Chinese traditional medicines. In fact, Hippocrates used arsenic for the treatment of skin ulcers dating back to 460 BC. In the late 18th century, arsenic was introduced in clinics to treat fever, CML, and many other diseases. Though, due to its toxicity, it was banned from used in the 20th century.

In 1992, the inorganic form of arsenic known as white arsenic, or ATO, was brought back to clinical trials for cancer remedy studies. ATO was found to exert dosage dependent effects on the human body. In high concentrations, ATO induces apoptosis, or sudden cell bursting. Under low concentrations, ATO tends to promote differentiation of only APL cells. The fact that ATO exerts selective therapeutic effects against APL but not against other leukemia subtypes suggests a link between its mechanism of action and the PML-RARα fusion product. It has been postulated, but never proven, that ATO induces change within the protein sequence of the APL gene; changes essential for the degradation of leukemic promyelocytic cells. **ATRA/ATO combination: A synergistic therapy**

When combined, ATRA and ATO produce a much longer survival in patients when compared with therapy of ATRA and ATO alone. It has been reported that with combination treatment, a shorter time to achieve complete remission is obtained as well as a profound reduction in the PML-RARα transcript. Since 2000, the diagnostic treatment for patients with APL is combination therapy.

Case Study(All pictures throughout this paper are from this case study)

Patient X, a 48 year old male, arrived at the ER at 6:35am on 4/7/2010 complaining of fatigue. The patient presented with localized petechiae on the skin surface. A CBC was performed and results were as follows: WBC: 11.6 K/μL, RBC: 2.76

M/ μL, Platelet Count: 25k/μL.These results are consistent with mild leukocytosis, anemia and thrombocytopenia, respectively. The peripheral blood smear was remarkable for abnormal promyelocytes. Auer rods were identified as well as prominent azurophilic granules within the cytoplasm. The platelets were markedly decreased. A Bone marrow biopsy was obtained and the results were as follows: The cellularity of the bone marrow was approximately 85%. A homogenous field of abnormal promyelocytes was observed. A bone marrow aspirate was also obtained and the smeared slides contained markedly increased amounts of abnormal promyelocytes containing large Auer rods. A bone marrow differential was performed with results concluding a myeloid: erythroid ratio of 13:8. The cellularity of the bone marrow was determined to be 85% homogenous. The MPO stain on the bone marrow cells was 99% positive and the chloroacetate esterase stain was 95% positive. Immunophenotyping was performed and results are summarized in Table 2. The coagulation studies were as follows: prothrombin time: 14.6s, D-dimer >6.0 FEU ug/ml, fibrinogen: 415 mg/dl, and aPartial Thromboplastin time: 22.7s. Analysis of these results shows that the patient is in acute DIC. Genetic analysis through FISH identified the presences of the fusion PML/RARα product. In all, these findings are morphologically consistent with Acute Promyelocytic Leukemia. Luckily, this patient was diagnosed in time and is currently on the ATRA/ATO treatment.

Summary

Acute Promyelocytic Leukemia has a unique clinical course with a specific chromosomal aberration the results in the formation of a fusion gene PML/RARα product. This chromosomal translocation plays a central role in APL leukemogenesis. Target therapy using ATRA and ATO share a common pharmacological activity of degrading this fusion product, therefore allowing differentiation to occur. Fundamentally,

APL is the first known disease that is clinically sensitive to differentiation therapy. It can be said with confidence that APL has gone from a highly fatal to a highly curable disease within the last sixty years and has mirrored the way to a basic understanding of all hematological neoplasms.