

2019

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Recommended Citation

Lamond, Alyssa and Tellier-Castellone, Theresa, "Acute Promyelocytic Leukemia: A Case Study Correlating Cytogenetic Abnormality with Prognosis" (2019). *Senior Honors Projects*. Paper 702.
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Acute Promyelocytic Leukemia:

A Case Study Correlating Cytogenetic Abnormality with Prognosis

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Abstract

Advancements in medical technology today have positively impacted the diagnosis, treatment, and prognosis of cancers. A diagnosis of acute promyelocytic leukemia (APL) has improved from having the poorest prognosis to one of the best. Acute promyelocytic leukemia is a malignant disease of hematopoietic tissue classified by WHO as leukemia with >20% blasts from the myeloid lineage, specifically promyelocytes. Determined in 1976, FAB classified AML subtypes M1-M7, with APL being M3. Specific characteristics classify the subtype of AML, with each resulting from a different genetic abnormality. APL diagnosis, treatment, and prognosis depends on the PML-RAR α fusion gene. Flow cytometry, karyotyping, and FISH are all methods done within the lab to determine this genetic abnormality. These molecular methods have been crucial in dictating targeted treatment options for patients and thus improving prognosis.

The purpose of the case study is to demonstrate the laboratory process by which APL becomes evident for the patient. By understanding the foundation of this disease, the importance of diagnostics comes to light. This case study focuses on a specific type of acute promyelocytic leukemia, the microgranular variant. Each step reviewed from physical examination to molecular methods contributes to prognosis. The goal in presenting this case study along with research findings is to emphasize the importance of diagnostic testing and the upcoming positive impacts the growing molecular lab world has on the patient population.

Index Terms

Acute promyelocytic leukemia, microgranular variant, PML-RAR α fusion gene, karyotyping, FISH, prognosis

Abbreviations

APL = acute promyelocytic leukemia; FISH = fluorescent in situ hybridization; WBC = white blood count; CBC = complete blood count; NOS = not otherwise specified; ATO = Arsenic trioxide; ATRA = all-trans retinoic acid; MPO = myeloperoxidase; SBB = Sudan black B; q = long arm

Objectives

1. Explain what acute promyelocytic leukemia is
2. Discuss the subtypes of acute myeloid leukemia and the features that characterize it
3. Describe the use of a CBC, stains, flow cytometry, karyotypes, and FISH to diagnosis APL
4. Interpret the mechanisms on treatment for acute promyelocytic leukemia and conclude why it is a success
5. Emphasize the relation between a cytogenetic abnormality and prognosis

Acute Promyelocytic Leukemia:

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Acute Promyelocytic Leukemia (APL) is a subclass of acute myeloid leukemia characterized by immature leukemic promyelocytes in the bone marrow and peripheral blood. Present in around 5-8% of AML cases, it is one of the best understood hematopoietic malignancies. Although APL has the most success with treatment when diagnosed, it can cause coagulopathy and death if not diagnosed promptly (Jaffe et. al, 2011, p. 676). What contributes to the understanding of APL is the known cytogenetic abnormality that occurs in most patients. The translocation between chromosome 15, the promyelocytic gene, and 17, the retinoic acid receptor gene, results in the fusion gene PML-RAR α . The effect the fusion gene has on the cells is inhibition of maturation, which leads to the proliferation of large numbers of atypical promyelocytes. The maturation process of these cells is at a halt (McKenna, 2000, p. 1252-1256). The case study below chronologically explains the procedures done to diagnosis a patient and treat effectively, with focus on the importance of the lab.

Case Study Overview

A 57-year-old female was admitted to the emergency room for symptoms of weakness, fatigue, and multiple bruises. The patient states she feels like she is coming down with the flu. Medical history revealed that she had only one procedure done in the past for an appendectomy. No current medical diagnoses were noted in her medical record. The attending ER physician stated the patient presented with pallor, along with petechiae on the arms and trunk. Samples were collected and sent to the lab for testing.

Morphological Diagnosis of APL

Blood collected from the patient was sent to the laboratories to perform the necessary testing. In hematology, the peripheral blood was analyzed and examined underneath the microscope. Results for the patient were abnormal, with characteristic thrombocytopenia and signs of anemia. Instead of typical leukopenia presenting in the patient, leukocytosis was noted (Harmening, 2009). Table 1 highlights important patient results that explain the presented symptoms and is suggestive for further testing. Examination underneath the microscope revealed predominating promyelocytes that were readily identified. The cells had a distinct morphological feature of bilobed nuclei with hypogranulation in the cytoplasm, as seen in figure 1 (Jaffe et. al, 2011, p. 677). In the bone marrow blood differential, 83% of cells were blasts with 12.1% of cells being lymphocytes. Both the MPO (myeloperoxidase) and SBB (Sudan black B) stains showed strong reactivity, and exposed the presence of granules (Harmening, 2009). Examination of the bone marrow biopsy indicated cellularity of approximately 70% with a monotonous population of immature cells. The bone marrow aspirate smear, seen in figure 2, also reveals the monotonous population of immature cells.

Table 1. Patient's significant laboratory results

Test	Patient Results	Normal Range
PT	33 sec	9-13 sec
PTT	41 sec	24-37 sec
Hemoglobin	8.3 g/dL	13.5-16.0 g/dL
Hematocrit	23.6%	37.0-47.0%
Platelets	21 x 10 ⁹ /L	150-400 x 10 ⁹ /L
WBC	11.9 x 10 ³ /L	4.8-10.8 x 10 ³ /uL
RBC	2.5 x 10 ⁶ /uL	4.2-5.4 x 10 ⁶ /uL

Patient presentation is consistent with characteristics of acute promyelocytic leukemia. Thrombocytopenia and leukocytosis indicate the patient has the microgranular variant of APL

(Baba et. al, 2018). This is confirmed by the peripheral blood smear. The peripheral blood characteristically exhibits increased promyelocytes that presented with no granules and bilobed nuclei. The strong reaction to both the MPO and SBB stain confirm that the cells are of the myeloid lineage and that the leukemia is AML. Also, due to blasts comprising 83% of cells (>20%), it is classified as AML per the WHO classification system (Ferreira, 2018). Due to being a myeloproliferative disorder, the bone marrow appears hypercellular. Since normal hematopoietic progenitor cells are being replaced by neoplastic blood cells, many cells are seen in the bone marrow aspirate and smear (Harmening, 2009). The monotonous population refers to the overproduction of the same cell, promyeloblasts.



Figure 1. Peripheral blood images displaying immature mononuclear cells, most with a cleft nucleus. The cells also appear to have an absence of granules.

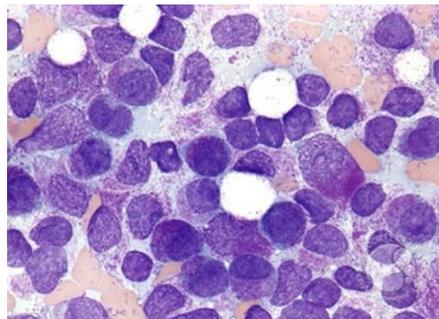


Figure 2. A bone marrow aspirate smear with a monotonous population of immature cells, presumably promyelocytes and blasts.

Flow Cytometry. Flow cytometry is an important diagnostic tool for immunophenotyping leukemia and lymphomas. Immunophenotyping is used to detect, characterize, and monitor abnormal cell populations (Harmening, 2009). By incubating the cells of interest with anti-human monoclonal antibodies conjugated to fluorochromes, markers specific to certain cell lineages are identified. Distinguishing the specific cell population present in a patient sample is highly useful in establishing an accurate diagnosis of AML. Flow cytometry also plays a role in monitoring the disease after the diagnosis has been made. It is used, “during clinical remission to monitor for minimal residual disease, and to detect relapse” (Harmening, 2009, p. 895). Due to being fast and highly sensitive, this technique is very useful for initial workup of samples to distinguish the type of leukemia the patient has.

The specimen received from the patient was incubated with the fluorochrome-labelled antibodies, lysed, fixed, and analyzed on the flow cytometer. Based on side scatter properties, the cells were gated using CD45. Results from patient’s histograms, figure 3, express the cell markers as the percentage of the blasts/blast equivalent forms. 100% of the cells expressed myeloid markers CD13/33, confirming the cells were of myeloid lineage. Expression of CD117 in 70% and CD34 in 25% of the cells indicate the presence of stem cells. Most of the patient’s cells show expression of CD64, and lack expression of HLA-DR. Characteristically, the microgranular variant of APL exhibits expression of CD13 and CD33, as seen in the patient’s histogram. Also, the immunophenotype of APL lacks expression of HLA-DR and CD34. However, if it is of the microgranular variant, it commonly demonstrates dim CD34 (Baba et. al, 2018). This explains the 25% of CD34 expressed in the patient’s cells. Based on the results in the peripheral blood smear, bone marrow biopsy and aspirate smears, cytochemical stains, and the

immunophenotype, the diagnosis for this patient is consistent with acute promyelocytic leukemia of the microgranular variant.

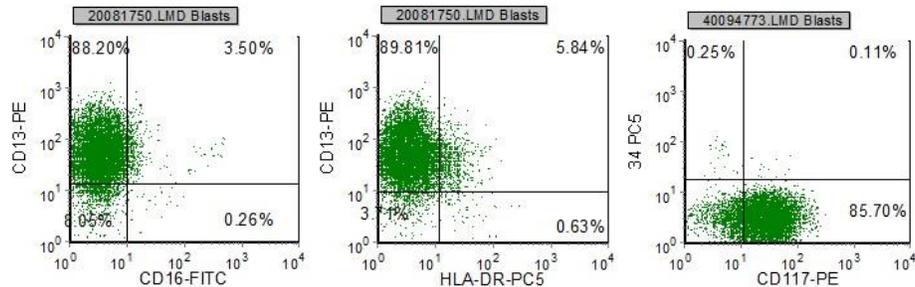


Figure 3. A-C. Flow cytometry identifies the cells expressing CD13 and CD117, with absence of markers CD16, HLA-DR, and CD34.

Molecular Diagnosis: Cytogenetics and FISH. Overall, “Cytogenetic testing should always be performed first in suspected APL patients” (Baba et. al, 2018, p. 43). Ruling in a cytogenetic abnormality is key in determining prognosis of the patient, along with relapse risk and overall survival. In APL, the fusion gene PML-RAR α results from a translocation between chromosomes 15 and 17. Using cytogenetic testing, detection of t(15;17) confirms the patients diagnosis (Swerdlow et. al, 2017).

The PML and RAR α genes serve an important role for normal hematopoiesis, “...with PML having both growth suppressor and proapoptotic activity and RAR α functioning as a transcription factor that mediates the effect of retinoic acid, which is necessary for normal myeloid, at specific response elements” (Adams, Nassiri, 2015, p. 1310). Patients with APL no longer have these functions due to the fusion of the genes. Adams and Nassiri explain:

In APL, the gain-of-function PML-RAR α fusion protein is believed to impair the normal growth suppressor and proapoptotic activity of PML and may prevent differentiation of

myeloid cells by repressing the target genes of retinoic acid, thus resulting in constitutive proliferation and inhibition of terminal differentiation (2015, p. 1310).

Promyelocytes are thus unable to proceed through normal myeloid differentiation.

Understanding the function of the genes and proceeding identification enables clinicians to diagnosis and treat rapidly.

Cytogenetic analysis for the patient included karyotyping and FISH. This aspect of diagnosing is essential and critical for the patient, providing information crucial for treatment and prognosis. Karyotyping involves staining of chromosomes during metaphase in order to distinguish structural rearrangements (Harmening 2009). The karyotype of the patient, figure 4 below, displayed the translocation of the PML gene at band q22 on chromosome 15 and the RAR α gene at band q21 on chromosome 17. Once t(15;17) is identified, the diagnosis is made and indicates a favorable prognosis. To confirm the presence of this translocation, FISH probes were used. FISH is a molecular method based on using a fluorescein probe to detect and localize specific DNA sequences in a metaphase or interphase chromosome. Once there is a source of light, the probe attached to a specific DNA sequence will fluoresce, rendering it visible (Harmening 2009). Using the specific probe t(15;17)(q22;q21), visualization of the translocation was confirmed by enhanced fluorescent microscopy.

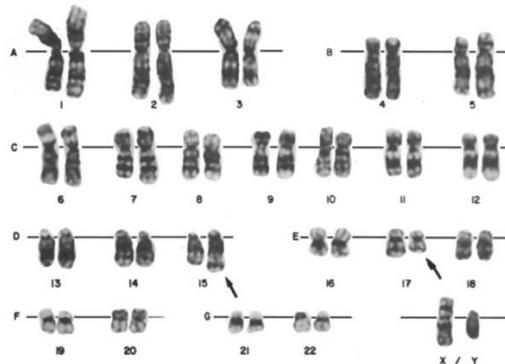


Figure 4. A karyotype displaying the translocation between the PML gene at band q22 on chromosome 15 and the RAR α gene at band q21 on chromosome 17, t(15;17)(q22;q21).

AML prognosis of subtypes without known cytogenetic abnormalities: Medical advancements in laboratory instruments and machines for leukemia and lymphomas over the past years have propelled patient treatment in the right direction. The technology available today provides a more depth understanding of these diseases, with the goal towards a potential long-term treatment. Although some subgroups of AML do have unique chromosomal abnormalities identified, others do not. Unknown cytogenetics of an AML leads to poor prognosis. As stated in the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, “with the possible exception of pure erythroid leukemia, the subgroups of AML, NOS, are not prognostically significant...” (Swerdlow et. al, 2017). With no knowledge of cytogenetics in an AML case, treatment is less targeted. Due to this, patient outcome includes poor prognosis, lower remission rate, short survival, and frequent early relapse (Ferreira, 2018). Although not every known cytogenetic abnormality is favorable, it provides information needed to potentially develop a targeted treatment to change the outlook. Other prognostic factors that contribute to a poor outlook for the patient include the presence of CD34 protein and/or the MDR1 gene product on the cells surface, over the age of 60, treatment-related AML, and a high WBC count at the time of diagnosis (American Cancer Society, 2018).

Treatment and Prognosis. Targeted therapy is the new and upcoming way of treating patients. By diagnosing based on molecular methods, more direct approaches are created for treatment. Acute promyelocytic leukemia is the most successful with treatment, therefore providing a better prognosis for the patient. With it previously being an AML with the highest mortality rates, to now being the least, it signifies the impact of targeted therapy (Adams, Nassiri,

2015). Paralleled by Julia Adams, MD, and Mehdi Nassiri, MD, "...despite the fact that APL has a high risk of coagulopathy and death if not treated quickly, the prognosis for treated patients able to achieve complete remission is better than that for any other category of AML" (2015).

Evidence of effectiveness provides comfort in the progression of cancer treatment for the future.

The agents used to treat APL are differentiating agents, which causes the cells to undergo maturation. Since the PML-RAR α fusion gene results in a defective retinoic acid receptor, the agents used are retinoids. The classic treatment of APL is the use of agents ATRA (all-trans retinoic acid) and ATO (Arsenic trioxide). According to the article *Acute Promyelocytic Leukemia: A Review and Discussion of Variant Translocations*, "classic APL is traditionally treated with retinoid-differentiating agents such as ATRA and ATO. Retinoids not only induce differentiation but also reduce the hemorrhagic complications of APL, whereas ATO causes both differentiation and apoptosis" (Adams, Nassiri). The combination of agents increases the effectiveness and encompasses the entirety of the disease. Understanding the baseline for normal physiological functions in cell maturation and differentiation provides information on how the agents work and target APL. The normal function of ATRA is "...to cause ligand-dependent conformational changes in wild-type RARA that induce dissociation of the corepressors SMRT or N-CoR so that cellular differentiation can proceed" (Adams, Nassiri, 2015, p. 1311). The corepressors are recruited by the action of RARA binding to retinoid acid response elements and promoters so they can function to mediate transcriptional repression. In the case of a PML-RAR α fusion gene, as exhibited in this patient, the "...concentration of ATRA is not high enough to induce the conformational change necessary for corepressor release, and cellular differentiation does not occur" (Adams, Nassiri, 2015, p. 1311). Due to this mechanism, the administration of ATRA is effective to increase concentration so that coactivators can initiate transcription and

facilitate APL blast differentiation. The success of this treatment can be explained by statistical findings in research, such that, “patients treated with ATRA for induction have an excellent outcome, with a 5-year DFS and OS of 69%, and may well be cured of their disease” (Tallman et. al, 2002, p. 4301).

Combining directed treatments has greatly improved prognosis for patients diagnosed with APL. The sooner treatment is started, the better the prognosis. Specifically, the cytogenetic findings of t(15;17) and the identification of PML-RAR α provide patients with the greatest prognosis, supporting the correlation between molecular discoveries and prognosis (Zahedipour et. al, 2017). Statistics from recent clinical trials show, “...that more than 90% of patients with classic t(15;17) APL are disease free and off treatment after 5 years” (Adams, Nassiri, 2015, p. 1312). Improvement in molecular findings for other subtypes of AML and leukemia and lymphomas in general will advance patient treatment and lead to overall treatment success.

Conclusion

All clinical laboratory findings were suggestive of acute promyelocytic leukemia of the microgranular (hypogranular) variant. The patient was promptly given ATRA and ATO treatment, along with starting anthracycline chemotherapy. Outlook on the future is positive due to the favorable prognosis she was given, and ability to enter complete remission is very possible.

Without molecular diagnostic methods, prognosis for acute promyelocytic leukemia patients would be far less positive. Molecular methods such as flow cytometry and FISH have drastically impacted the medical field in the best way possible. More patients are living longer lives due to advancements in detection of cancers. Thus, the treatment for cancer is improving.

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