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Research Article

Acute Promyelocytic Leukemia (APL) - Biology, Diagnosis and Prognosis

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Abstract

Acute promyelocytic leukemia (APL) is one of the medical emergencies in malignant hematology, wherein, high index of suspicion and early diagnosis is paramount. In 90-95% cases this malignancy results from a balanced translocation, commonly t(15;17)(q22;q12-21), which leads to the fusion of the promyelocytic leukemia gene (PML) with retinoic acid receptor alpha gene (RARA). Other rare cases (10-15%) which arise due to cryptic or complex rearrangements lack this classical translocation. Genetic diagnosis becomes essential in such patients as these patients may have atypical clinical presentations, require a different approach to treatment and have different prognosis in long run. APL on morphology has classical "buttock shaped" abnormal promyelocytes with faggots - However this classical morphology may be absent in variants. Immunophenotypically also - variants may have a deviant phenotype. Crisp management of early complications can save precious lives. Development of new targeted molecules have changed the course of APL treatment.

Keywords: Promyelocytic Leukemia; Retinoic Acid Receptor; ATRA

Abbreviations

APL: Acute Promyelocytic Leukemia; HDAC: Histone Deacetylase; NB: Nuclear Body; PLZF: Promyelocytic Leukemia Zinc Finger; PML: Promyelocytic Leukemia; RA: Retinoic Acid; RAR: Retinoic Acid Receptor; RARE: Retinoic Acid-Responsive Element; RXR: Retinoid X Receptor; FLT 3 - ITD: FMS Like Tyrosine Kinase 3 - Internal Tandem Duplication

Introduction

Acute promyelocytic leukemia (APL) is amongst one of the highly curable and responsive disease in hematological malignancies. It is one of the subtypes of acute myeloid leukemia (AML) with a defined clinical course and a distinct biology which separates it from other AML subtypes [1]. This distinction is essential for clinician not only because the chemotherapy used is different from other subtypes of AML but mainly because of the relative common occurrence of many life threatening complications. Despite prompt diagnosis and careful management of potential complications associated with institution of therapy, peri - induction mortality is significantly higher than in other types of AML, reaching upto 50% as reported by few series. Hence, this subtype is the one which should be identified early and monitored closely in order to avoid any untoward outcomes.

Biology and pathogenesis of APL

Erstwhile known commonly as AML M3 (as per FAB classification) - the current WHO 2016 classification of haematological malignancies places this category of AML

under AML with recurrent cytogenetics abnormalities (Acute Promyelocytic leukaemia with PML - RARA) as the entity is defined by its genetic lesion. It comprises 8-10% of all adult AML cases with a median age of 37yrs. APL is generally uncommon in paediatric populations, although rare cases have been reported. It is also not generally preceded by myelodysplastic syndrome emphasizing its distinct biological origin than other AML subtypes. Secondary APL (sAPL) is defined as APL emerging after chemotherapy and/or radiotherapy used for malignant or non malignant conditions and represents about 10% of the newly diagnosed APL cases. Patients presenting with APL post chemotherapy (topoisomerase II inhibitors) behave similar to de novo APL in terms of disease progression/complications unlike other secondary AMLs.

	Translocation	Frequency	Function gene	Sensitivity to chemotherapy
1.	t(15;17)	95%	PML RARA	Yes
2.	t(11;17)	<5%	PLZF RARA	No
3.	t(5;17)	<1%	NPM RARA	Yes
4.	t(11;17)	<1%	NuMA RARA	Unknown
5.	t(17;17)	<1%	STAT 5B RARA	Unknown

Table 1: Chromosomal translocations common in APL.

APL is the best example of comprehensive medicine with increasing understanding of molecular and therapeutics in its biogenesis [2]. Of the five common translocation noted in APL(as listed in table 1), the most common one is t(15;17) (q24.1;q21.2) seen in almost 90-95% cases. The translocation places the 5' portion of PML gene adjacent to 3' portion of RARa gene and a fusion protein (PML RARA) is formed. The wild type RARA is a nuclear receptor and binds to many Retinoic acid response elements (RARE) in presence of retinoic acid (RA). These RARE are commonly found in the promoter regions of many genes, especially, the genes responsible for myeloid differentiation eg G CSF, neutrophil granule protein, cell surface adhesion molecule and other regulators of apoptosis and transcription. In absence of RA, wild type RARA presents as a heterodimer on RARE and binds many co repressors eg SMRT, N -COR, mSin 3 and histone deacetylase (HDAC). When exposed to RA in physiologic concentrations - these co repressors are released and activation of transcription occurs.

Wild type PML protein is a tumour suppressor gene and is generally localized in subnuclear domains called nuclear bodies (NB). Its main function is apoptosis and growth suppression. The protein encoded by PML - RARA fusion transcript is delocalized from nuclear bodies to outside in a microspeckled pattern. This fusion product (PML RARA) also binds to RARE as strongly as wild RARA but the physiologic concentrations of RA (10^{-9}) is not able to release the co repressors when transcription activation is required. However, raised concentrations (10^{-6}) of ATRA is able to release these co repressors and induce transcriptions. This forms the basis of pathogenesis of APL wherein the fusion product hinders the differentiation and maturation of myeloid cells leading maturation arrest and accumulation of abnormal promyelocytes. Exposure to ATRA induces differentiation and maturation due to release of the co repressors and appearance of neutrophils.

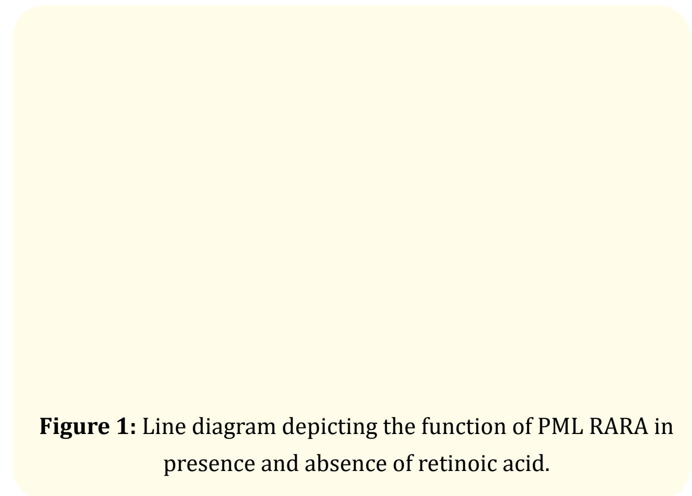

Figure 1: Line diagram depicting the function of PML RARA in presence and absence of retinoic acid.

Initial studies proposed that the observed differentiation of APL cells upon RA treatment results from gene transactivation by PML-RARA itself, evoking the re-expression of the previously silenced genes. Yet, the central role of PML-RARA degradation in APL response to treatments suggests alternative models by which the loss of the fusion protein would clear the way for other transcription factors to bind the promoters of their target genes and regain control over their transcription. These could include RA-bound RARA complexes that are capable of reinitiating the process of myeloid differentiation as shown in figure 1.

However, not all other complex/ cryptic translocation have such straightforward mechanism. PLZF- RARA or t(11;17) fusion product forms a second binding with co repressors on PLZF portion in addition to the original one on RARA portion. ATRA induces release of co repressors from RARA portion but PLZF portion binding is unaffected hence patients with this translocation do not respond to ATRA in isolation but need HDAC inhibitors too. The other translocations as mentioned in table 1 are rare, with complex mechanism and their treatment response profile is also not so well known at present [3].

Although t(15;17) or other translocation involving chr 17, is the defining cytogenetic abnormaility in APL, additional chromosomal abnormalities are commonly seen in upto 30% of patients. The common ones are trisomy 8, isochromosome 17, chr 9 or chr 3 abnormalities. However, these additional chromosomal abnormalities do not seem to affect the overall prognosis of patient. Co - existing molecular mutations like FLT 3 - ITD are also seen in high frequency (30%).

Clinical presentation

Like any other leukemia, the symptoms associated with APL are either non specific or arise due to cytopenias. Fever, fatigue, bleeding symptoms are commonly reported symptoms. Some patients get the medical attention due to pancytopenia which is a very common presentation in APL. Hepatosplenomegaly if present is usually small in size due to early presentation otherwise. Extramedullary infiltrations (gum hyperplasia, chloromas) are also rare at presentation. Rarely patient is brought to medical attention by associated coagulopathy [4].

Diagnosis

The laboratory has a very important role in guiding the clinician in these cases towards correct diagnosis. The complete blood count is generally amongst the first laboratory tests and is almost always abnormal. Rapid confirmation on peripheral smear usually leads towards more specific tests faster. The tests are discussed under morphology, flowcytometry, genetic tests and other contributory tests.

Morphological diagnosis

As a dictum, a peripheral smear examination is a must in any case of cytopenias. Though pancytopenia is a common finding, WBC may be high in 20% cases. Thrombocytopenia is universal. Peripheral smear usually often contains the characteristics cells - abnormal promyelocytes - in varying concentration. They are larger than normal promyelocyte with bilobed (buttock shaped) nuclei and heavily granular dusty and dusky cytoplasm with auer rods. The pathognomic “Faggots” are found when looked for especially in tail areas of smear. The other morphological variants like - microgranular variant (M3V), Hyperbasophilic variant and Zinc finger/RARE usually pose diagnostic difficulties as they lack characteristic morphology and auer rods (Figure 2). Cytochemistry helps in early diagnosis by demonstration of strong burst positivity of myeloperoxidase on MPO staining. Sudan black B and NSE staining pattern also helps in differentiating from monocytic AML [5]. Bone marrow is generally hypercellular demonstrating similar abnormal promyelocytes. Fibrosis or necrosis is genrally not common findings. Immunofluorescence can be utilised to demonstrate the microspeckled pattern of PML RARA protein distribution using anti PML monoclonal antibodies if available.

Figure 2: Photomicrographs of morphological variants of APML - A. classical type showing hypergranular promyelocytes with faggots B. microgranular variant showing hypogranular bilobed promyelocytes with Auer rods C. Hyperbasophilic variant with megakaryoblast like promyelocytes D. M3r variant showing promyelocytes with regular nuclei and pseudo-Pelger cells (LG, 100X) (Picture courtesy: Acta Haematol 2019;141:232-244).

Immunophenotype

The majority of classical (hypergranular) APL show characteristic features on flowcytometry. The most common feature of side scatter property was the absence of blasts in the usual blast window and high side scatter giving it a "tear drop pattern' to it (Figure 3). Classic APL is Immunophenotypically characterised by lack of expression of HLA-DR, CD11a, CD11b, CD18, positive CD117, negative or weakly positive CD15 and CD65, negative CD34, often positive CD64, variable (heterogeneous) CD13 and bright CD33. (Figure 3) Hypogranular (microgranular) variant of APL differed from classical APL by low SSC and frequent co-expression of CD2 and CD34. Rare cases of APL may show a mixture of neoplastic cells (low SSC/CD2+/CD13+/CD33+/CD34+/CD117+) and prominent population of benign granulocytes/maturing myeloid precursors (high SSC/CD10+/-/CD16+/-/ CD117-). In cases where morphology is confusing with AML M4/5 - CD 9 serves as very distinguishing marker. In almost 90% of cases CD9, CD2, and CD34, CD56 positivity is seen with the specific exclusion AML-M4/M5. Some studies have found correlation between positive CD 2, CD34 and CD56 with poor prognosis and higher incidences of complications and relapse. CD9, however, was significantly associated with good prognostic outcome in APL [6].

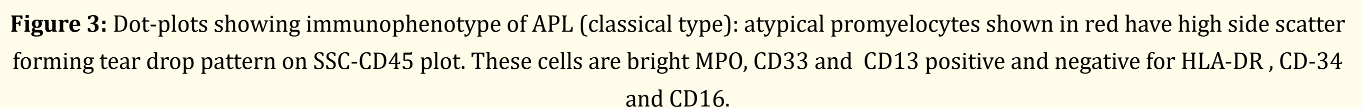

Figure 3: Dot-plots showing immunophenotype of APL (classical type): atypical promyelocytes shown in red have high side scatter forming tear drop pattern on SSC-CD45 plot. These cells are bright MPO, CD33 and CD13 positive and negative for HLA-DR , CD-34 and CD16.

Genetic testing

A rapid confirmation of genetic diagnosis is mandatory and should ideally be performed on Bone marrow sample. The identification of APL specific genetic lesion can be made by conventional karyotyoing, Flourescence in situ hybridization (FISH), reverse transcriptase polymerase chain reaction (RT PCR) or real time PCR. While all the above methods are equally specific, sensitivity varies amongst them with cytogenetic analysis being most prone to false negativity. other (karyotyping, FISH) methods cannot substitute for RT-PCR or RQ-PCR, which should always be run in parallel, as the only technique allowing definition of the type of PML/RARA isoform and quantification for subsequent MRD evaluation. specific break point cluster region (bcr1, Bcr 2, bcr 3) identification can be done only by PCR methodology which may have a bearing on thrombotic complications (most commonly seen in bcr2) [7].

Figure 4: Dual colour- dual fusion FISH probe showing PML/RARA loci as indicated by one separate orange signal one separate green signal and two orange/green fusion signals.

Other lab tests

The supportive measures recommended to monitor/treat the coagulopathy are very important. Platelet counts and routine coagulation parameters, including prothrombin time, activated partial thromboplastin time, and thrombin time, as well as levels of fibrinogen and fibrinogen-fibrin degradation products should be monitored at least daily and more frequently if required. Transfusions of fibrinogen and/or cryoprecipitate, platelets, and fresh-frozen plasma should be given immediately upon suspicion of the diagnosis, and then daily or more than once a day if needed, to maintain the fibrinogen concentration above 100 to 150 mg/dL, the platelet count above 30,000- 50,000/micro L, and the international normalized ratio (INR) below 1.5. this should be continued during induction therapy until disappearance of all clinical and laboratory signs of the coagulopathy.

Management

Patients with APL should be managed by an experienced team in centres with documented rapid access to genetic diagnosis, a broad range of blood products well as ATRA, ATO, and chemotherapy. ATRA should be initiated immediately once APL is suspected; if the diagnosis is not supported by genetic or molecular data, it should be discontinued. For patients presenting with low WBC count (10x3 10^9/L), administration of other antileukemic agents such as ATO or chemotherapy may be delayed until the genetic diagnosis is confirmed; however, in patients with leukocytosis (ie, WBC count >10,000/micro L), chemotherapy should be started without delay even if the diagnostic molecular results are still pending. Common complications (differentiation syndrome, fluid overload, coagulopathy and relapse) as anticipated should be monitored and treated on priority. As a general rule, treatment of patients with ATRA-sensitive variants should include this agent in combination with anthracycline-based chemotherapy, whereas in those with ATRA-resistant variants, the addition of ATRA is less attractive and management should consist of AML-like approaches.

Prognosis

Despite tremendous progress, APL still remains associated with a high incidence of early death due to the frequent occurrence of an abrupt bleeding diathesis. This haemorrhagic syndrome more frequently develops in high-risk APL patients, currently defined as those exhibiting $>10 \times 10^9$/L WBC count at presentation [8]. In addition to high WBC count, other molecular and immunophenotypic features have been associated with high-risk APL. In this section, we briefly discuss the various prognostic factors in APL.

Cellular prognostic marker (CD 56, CD 2, CD 34)

Aberrant CD56 expression may be observed on immature granulopoietic cells under conditions of increased proliferation

or of increased growth factor stimulation. In APL patients, WBC counts at diagnosis were higher among CD56+ compared to CD56− patients. Also, CD56+ and CD 2 + APLs more frequently displayed bcr3 iso- form and was shown to be an independent prognostic factor for relapse. Low or absent CD34 expression is considered a typical feature of the APL immunophenotypic profile, together with absent HLA-DR expression. However, a small proportion of APL patients express CD34 in their blasts at diagnosis. The latter has been associated with leukocytosis, hypogranular morphology, and/or the S-form of the PML-RARA transcript

WBC count at presentation

Based on established consensus, WBC ≥10 × 10^9/L is considered to convey higher risk of both early death and relapse. In this context, Burnett and co-workers reported that WBC count at diagnosis represents the only factor influencing APL outcome in patients receiving ATRA and chemotherapy. Patients with WBC counts ≥10 × 10^9/L had an inferior remission rates, disease- free survival and overall survival rate and an increased incidence of early mortality and relapse compared to patients with WBC counts <10 × 10^9/L. Low- and intermediate-risk APL (differentiated by platelet counts above and below 40 × 10^9/L) also referred to as standard-risk APL are defined by a WBC count of equal to or less than 10,000/μL. A presentation WBC count greater than 10,000/μL represents high-risk APL.

High Risk APL	WBC > 10,000 / micro L	Irrespective of platelet count
Standard Risk APL	WBC < 10,000/ micro L	Platelet count >40,000 (intermediate risk) <40,000 (low risk)

Table 2

Additional abnormalities (cytogenetics/molecular)

It is well known that activating internal tandem duplication (ITD) mutations in the Fms-Like Tyrosine kinase 3 (FLT3) gene (FLT3-ITD) are associated with poor outcome in AMLs, but their prognostic significance in APLs has remained controversial. Mutations of the FLT3 gene have been detected in 30-40% of APLs: 20-30% consist in ITDs occurring at the level of the juxtamembrane domain of the gene (FLT3-ITD); 8-12% are activating point mutations occurring in the loop of the tyrosine kinase domain 2 (TDK2) of FLT3, mainly located at the level of the D835 amino acid residue. The presence of FLT3-ITD in APL has been associated with - Increased occurrence of thrombotic events, Increased white blood cell counts (WBC), high LDH levels, Immature cell phenotype (CD34 + CD+ and M3V morphology) and short/bcr3 isoform.

Interestingly, a greater number of high-risk APL patients carried additional mutations as compared to intermediate- and low-risk patients; more importantly, patients with mutations of the epigenetic modifier genes (DNMT3A, MLL, IDH1, IDH2, and TET2) displayed a significantly reduced remission and survival rates, compared to patients lacking these mutations. Patients with additional abnormalities more frequently had coagulopathy, lower platelet counts, but none of them could be identified as independent risk factors for relapse.

Genetic polymorphism

A functional variant in the core promoter of the CD95 death receptor gene (a common G > A polymorphism at position -1377) was associated with a worse prognosis in APL patients. Other genetic polymorphism under research affecting APL biology/ prognosis includes TP73 gene transcript alternate splicing polymorphism affecting response to therapy.

The analysis of the available data indicates that, in spite the numerous prognostic biomarkers identified, the stratification of APL risk according to Sanz stratification based on WBC and platelet counts remains the most reliable and validated way to rapidly identify high-risk APL patients [9]. It is therefore important to rapidly identify these patients by comprehensive clinical, immunophenotypic, and molecular characterization. Integrating genotypic and phenotypic markers help in development of optimised treatment [10].

Conclusion

Acute promyelocytic leukemia is a distinct subset of AML characterized by peculiar molecular, morphologic, biologic, and clinical features. Early diagnosis saves life. Risk assessment in APL management requires distinguishing prognostic factors associated with early death and increased probability of relapse. Despite the progress in treatment field, induction mortality remains one of the main obstacles in APL therapy.

Key Points to Remember

- Acute Promyelocytic leukaemia with PML - RARA is defined by its genetic lesion
- It is uncommon in paediatric age group or post chemotherapy and certainly not described as post MDS leukemia
- Common translocation include- t(15;17) -95% cases, t(11;17 t(5;17 t(17;17) and other rare ones
- Morphological variants include - classical type, microgranular variant (M3V), Hyperbasophilic variant and Zinc finger/RARE type
- Classic APL is Immunophenotypically characterised by lack of expression of HLA-DR and CD 34. Strong positive MPO, and CD117, often positive CD64, variable CD13 and bright CD33
- Microgranular variant is associated with invisible granules, CD 2 positivity, short iso form of PML RARA and higher chances of thrombotic events
- RT PCR/RQ PCR for PML RARA have important role not only in diagnosis, prognosis but also in MRD monitoring
- WBC count > 10,000/micro L is the sole important prognostic factor affecting survival.
- ATRA should be initiated on first suspicion of APL- can be discontinued if not supported by genetic studies.
- Additional abnormalities like trisomy 8, isochromosome 17, chr 9 or chr 3 abnormalities or FLT 3 ITD mutation do not affect overall prognosis.

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