

Study of cytogenetic abnormalities in 190 cases of acute promyelocytic leukemia with its morphological and cytochemical correlation at a tertiary-care cancer institute

Binal D Vaghani¹, Hardik R Shah², Heming Agrawal¹, Shilpa Singal¹, Dhaval Bamanian³, Hansa Goswami¹

¹Department of Pathology, BJ Medical College, Ahmedabad, Gujarat, India.

²Department of Medicine, GMERS Medical College, Sola, Ahmedabad, Gujarat, India.

³Department of Pathology, Gujarat Cancer and Research Institute, Ahmedabad, Gujarat, India.

Correspondence to: Binal D Vaghani, E-mail: binal9913397576@gmail.com

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Abstract

Background: Acute myelogenous leukemia (AML) is a malignant disorder of the bone marrow, where a maturational arrest in blood cell progenitors results in the failure of normal hematopoiesis. Acute promyelocytic leukemia (APL) is a subtype of AML with a defined clinical course and a biology that is distinct from the other forms of AML.

Objective: To detect cytogenetic abnormalities in patients diagnosed with APL and to detect APL-like cases.

Materials and Methods: A total of 190 cases were reviewed. Bone marrow smears were studied and morphological diagnosis was made. Genotype was analyzed using G-banding.

Result: In ark of the 190 cases, it was agreed that the morphologic diagnosis was FAB M3 (either hypergranular form or variant form). Of these, 142 cases had confirmed APL, and they responded to all-*trans* retinoic acid (ATRA). Of these cases, 101 (53.1%) had t(15;17). Of the remaining 103 cases, six was positive fusion product PML/RAR α , and five cases revealed a t(11;17). The remaining 48 (25.2%) cases showed chromosomal abnormalities as del 16q, trisomy 8, t(8;21), del 8q, add 15q, +10, and ins [1q]. However, these cases presented with morphological and immunophenotype characteristics of APL; so, they were called as APL-like leukemia.

Conclusion: Our study shows that many other chromosomal abnormalities other than t(15;17) can show similar morphologic, cytochemical, and immunophenotypic characteristics of APL.

KEY WORDS: Cytogenetics, morphology, promyelocytic leukemia

Introduction

Acute myelogenous leukemia (AML) is a malignant disorder of the bone marrow, where a maturational arrest in blood cell progenitors results in the failure of normal hematopoiesis. Acute promyelocytic leukemia (APL) is a subtype of AML with

a defined clinical course and a biology that is distinct from the other forms of AML. Morphologically, the most common form of APL can have a characteristic appearance, because the bone marrow is effaced by heavily granulated cells with folded, twisted nuclei. Biologically, the cytogenetic changes define the syndrome, and the molecular consequences of the chromosomal changes found in APL play a critical role in leukemogenesis.^[1] APL [AML with t(15;17)(q22;q21)] is an acute myeloid leukemia, in which abnormal promyelocytic predominate. Both hypergranular or "typical" APL and microgranular (hypogranular) types exist.^[2] Characteristic cells containing bundles of Auer rods (faggots) randomly distributed in the cytoplasm are almost invariably present in the bone marrow and sometimes in the peripheral blood. The cytoplasm of the cells that contain faggots is often clear and pale staining but

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may also contain azurophil granules. The cytochemistry of both forms of M3 shows characteristic strong positivity for peroxidase or Sudan Black B and for chloroacetate esterase. The bundles of Auer rods are recognized easily with the latter stains but not the former.

A unique chromosomal translocation that defines M3 is t(15;17)(q22;q21).^[3] This translocation results in the fusion of the promyelocytic leukemia gene “PML” and the retinoic acid receptor (RAR α) gene.^[4] With the use of conventional cytogenetics, the t(15;17) can be detected in majority of the cases with M3 at the time of diagnosis. Probably, all cases can be detected by either the application of fluorescence *in situ* hybridization (FISH)^[5,6] or by molecular analysis.^[7,8]

In addition, Gallagher et al.^[9] have described molecular variants of the fusion product—the long or L form (PML exon 6 to RAR α exon 2; the variable or V form), exon 6 coding nucleotides to RAR α exon 2, and the short or S form (PML exon 3 to RAR α exon 2). Neither of the two major types (S and L forms) have a prognostic significance although the microgranular variety of M3 is more commonly seen with the S form.^[10]

In 1993, Chen et al.^[11] described a fusion product between a novel zinc finger gene and the retinoic acid receptor with t(11;17)(q23;q21) in a patient with APL. Licht et al.^[12] reported on five additional “M3-like” cases with morphology “in a small morphologic spectrum with features intermediate between M2 and classical M3 AML.” The gene fusion product was referred to as promyelocytic leukemia zinc finger (PLZF)—RAR α . None of the patients responded to all-*trans* retinoic acid (ATRA). The published photomicrographs reveal myeloblasts with heavy granulations but no faggot cells or nuclear features of M3, although the report mentions “faggots” and/or Auer rods in three of four cases.

In 1996,^[13] a second novel translocation t(11;17)(q13;q21) was identified in a survey of patients diagnosed morphologically as “APL” (FAB M3). The fusion product described was nuclear mitotic apparatus (NuMA)—RAR α . Finally, a different translocation, t(5;17)(q32;q12) associated with the nucleophosmin (NPM)—RAR α fusion gene product was described.^[14] In a subsequent article, Redner et al.^[15] demonstrated maturation to neutrophils in cell culture treated with ATRA.

To further clarify this important observation, members of the FAB Co-operative Group held a workshop in June 1996 in London, UK. In addition to the FAB members, Dr Cheryl Willman, Professor of Pathology at the University of New Mexico Cancer Center and Chair of the Leukemia Biology Committee of the Southwest Oncology Group (SWOG), attended.

Materials and Methods

Cases of both classical M3 and those with different cytogenetic findings but resembling M3 were assembled. Romanowsky-stained slides were examined after being coded. The blasts were examined for size, chromatin pattern, nuclear features, cytoplasmic features, and the presence of Auer rods. On the basis of morphologic features, the reviewers gave an impression (AML-M3 or AML-M3-like). The

cytochemical (Sudan Black and periodic acid Schiff), immunophenotypic, karyotypic, and molecular features (if available) were also reviewed. On the basis of morphological assessment, cases were diagnosed as classical M3, M3 variant, and not M3.

Flow cytometric data for the cases were reviewed retrospectively. Analysis was performed initially on the peripheral blood and/or bone marrow aspirate specimens. Antibody combinations selected for review included CD45, CD34, CD14, CD13, CD33, HLA-DR (Becton Dickinson), and CD56 (Coulter).

For karyotype analysis, cells from unstimulated peripheral blood or bone marrow aspirate specimens were arrested at metaphase with colchicine. Chromosomes were stained by the G-banding method. The chromosome number was determined by microscopic analysis, and the cells were examined for the presence or absence of detectable structural rearrangements. Karyotypes were prepared from computer-assisted images of the metaphases.

FISH was also performed using a PML/RAR α dual-color translocation probe.

A total of 238 cases were reviewed over a period of 30 months. Molecular analysis by FISH was performed in 160 cases for further confirmation.

Result

The patients in this study included 132 male subjects and 106 female subjects, with a mean age range at diagnosis of 1–60 years. At diagnosis, all patients revealed a variety of nonspecific symptoms, including cough, nasal congestion, arthralgia, fatigue, ecchymosis, and fever.

At diagnosis, most patients showed moderate to marked leukocytosis [mean WBC count, 40,860/ μ L (40.8×10^9 /L)]. Most patients were mildly to moderately anemic at initial examination [mean hemoglobin concentration, 7.5 g/dL (75 g/L); range, 1.5–15.8 g/dL (15–158 g/L)]. Thrombocytopenia was noted in all study patients [mean platelet count, 20×10^3 / μ L (20×10^9 /L)]. The APL and APL-like cases were characterized by predominantly intermediate-sized blasts with fine to mildly clumped chromatin, irregularly shaped nuclei with frequent folding and bilobation, and granulated basophilic cytoplasm [Figure 1]. Auer rods were noted in the blasts of 32 cases. No cases showed Pelger-like cells. No morphologic parameters such as blast size, chromatin pattern, nuclear shape, or presence of cytoplasmic vacuoles differentiated APL-like leukemia from AML-M3v. Strong block staining for Sudan Black was noted in 182 of 190 cases; no difference in staining intensity was identified between AML-M3 and AML-M3-like leukemia.

There were 190 cases in which it was agreed that the morphologic diagnosis was FAB M3 (either hypergranular form or variant form).^[16,17] Of these, 142 cases showed confirmed APL and responded to ATRA. Of these cases, 101 revealed t(15;17). Of the remaining 89 cases, six was positive fusion product PML/RAR α and five cases showed a t(11;17). The

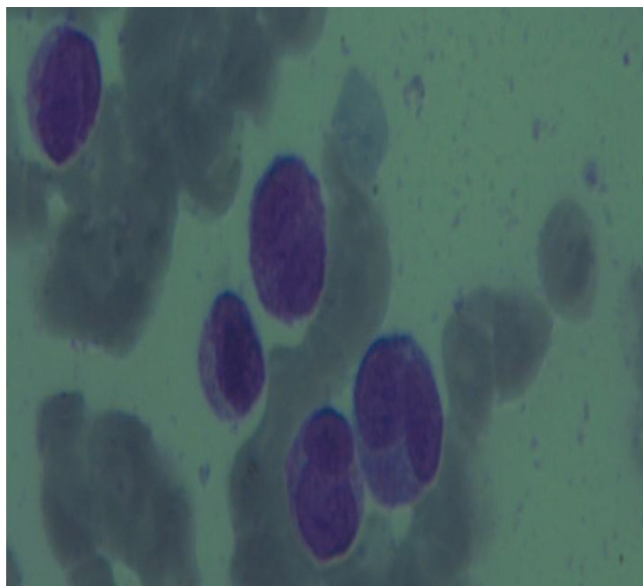


Figure 1: Bone marrow showing bilobation and granulations.

Table 1: Other chromosomal abnormalities

Chromosomal abnormality	No. of cases
45,X(-X)del 8q/46,XX	1
46, XX	16
46,XY	16
46,XX,i[17q]/46,XX	3
46,XX,ins[1q]/47,XX,?+19,ins[1q]	1
46,XX,t(8,21)(q22;q22)	2
46,XX[6]/92,XXXX,[1]/91,XXX[1]/45,XX,-13[1]/45,XX,21[1]/44,XX,-20,-20[1]	1
46,XY,add[15q]	1
46,XY,del(16q)	1
47,XX,+8	3
47,XX,+10/46,XX	1
48,XY,+8,+21,del(16)(q21)/47,XY,+8,del(16)(q21)	1
49,XY,+8,+21,+19,t(9,22)(q34;q12)	1

remaining 48 cases showed AML-M3-like leukemia. These 48 patients revealed chromosomal abnormalities as shown in Table 1.

In this study, in the absence of t(15;17) or PML/RAR α on FISH, 13.4% (32 cases) were diagnosed to be in remission with karyotype 46, XY or 46, XX.

One case showed del 16q and was diagnosed as AML M4 as patients with inv/del(16) (p13q22)/del(16) (q22)/t(16;16) (p13;q22) usually correspond to the subclass of AML M4, with a specific abnormal eosinophil component and is considered as a distinct entity in correlation with these specific chromosomal abnormalities. These cases of AML M4 are referred as AML M4E0.^[19]

Table 2: Additional chromosomal abnormalities along with t(15;17)

Additional chromosomal abnormality	No. of cases
+21	1
i[17q]	4
+8	6
del 1q	1
del 11	1
+12	1
del 9q	1
add 10q	1

- Four cases revealed trisomy 8. Trisomy 8 either alone or associated with other aberrations is the most frequent abnormality in patients with APL.^[20]
- Three cases revealed i[17q] and were diagnosed as myelodysplastic syndrome.
- Two cases were diagnosed as FAB AML M2 having t(8;21).

Other additional chromosomal abnormalities detected were del 8q, add 15q, +10, and ins [1q].

Furthermore, in this study, 15 patients showed additional chromosomal abnormalities in addition to t(15;17), as shown in Table 2. Trisomy 8 was the most frequent additional abnormality.^[20] This study also presented 6 cases with trisomy 8, followed by 4 cases of i[17q], as the most frequent additional abnormality.

Discussion

APL is characterized by a proliferation of abnormal promyelocytic. The disease comprises about 5% to 10% of all AMLs^[21] and is divided into the classic (hypergranular) and microgranular (hypogranular) types. The classic form of APL is characterized by t(15;17)(q22;12) or the presence of the PML/RAR α transcript. Three alternative translocations associated with APL also have been characterized: t(11;17)(q23;q21) (PLZF/RAR α), t(5;17)(q35;q21) (NPM/RAR α), and t(11;17)(q13;q21) (NuMA/RAR α).^[22]

Because patients with APL benefit from treatment with retinoid or arsenic compound therapy^[23,24] and have an increased risk of DIC and hemorrhage, the correct classification of APL is important. Definitive diagnosis is based on cytogenetic and molecular tests; these studies are highly technical and expensive, and results, often, are not available in a timely manner. Pathologists often are faced with the dilemma of classifying APL based only on morphologic, cytochemical, and immunophenotypic findings. Our study confirms that there is considerable difficulty distinguishing AML-M3v from APL-like leukemia based on these parameters.

Because morphologic, cytochemical, and immunophenotypic studies, often, are nonspecific in these leukemias, cytogenetic studies are necessary for accurate classification. Cytogenetically detectable rearrangements are identified

in about 70% to 80% of cases.^[25] In this study t(15;17) was detected in 53.1% cases that correlated with morphological and immunophenotypic diagnosis of APL. Additional studies such as FISH or RT-PCR may increase detectability of the translocation or transcript to almost 90% of cases.^[26] Sainty et al.^[27] undertook a morphologic, immunophenotypic, cytogenetic, and molecular review of a series of cases referred to as APL lacking t(15;17) and compared them with a control group of cases of APL with t(15;17). The majority of the APL cases that did not demonstrate t(15;17) revealed underlying rearrangements of the PML/RAR α transcript by FISH or RT-PCR.^[27]

Conclusion

Our study shows that many other chromosomal abnormalities other than t(15;17) can show a similar morphologic, cytochemical, and immunophenotypic characteristics of APL. In such cases, karyotype analysis, FISH, and RT-PCR must be conducted to rule out APL. This APL-like cases constitute a heterogeneous group of population. The strength of the study is that cytogenetic was useful to detect 50%–55% cases of morphologic APL, which was strengthened by molecular study up to 80%–90%. But limitation of this study is that there is a considerable difficulty distinguishing AML-M3v from APL-like leukemia based on the morphological and cytochemical parameters.

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