



Jornal Brasileiro de Patologia e Medicina Laboratorial

ISSN: 1676-2444

ISSN: 1678-4774

Sociedade Brasileira de Patologia Clínica; Sociedade Brasileira de Patologia; Sociedade Brasileira de Citopatologia

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Jornal Brasileiro de Patologia e Medicina Laboratorial,
vol. 55, no. 6, 2019, November-December, pp. 580-597

Sociedade Brasileira de Patologia Clínica; Sociedade Brasileira de Patologia; Sociedade Brasileira de Citopatologia

DOI: 10.5935/1676-2444.20190052

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Acute promyelocytic leukemia: evaluation of diagnostic tests from 2000 to 2018 in a public hospital

Leucemia promielocítica aguda: avaliação dos testes diagnósticos entre o período de 2000 e 2018 em um hospital público

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ABSTRACT

Introduction: Acute promyelocytic leukemia (APL) is caused by t(15;17)(q24;q21) translocation, which product is the fusion oncoprotein *PML-RAR α* (promyelocytic leukemia-retinoic acid receptor alpha). The morphology of leukemic promyelocytes is usually characteristic, with the presence of faggot cells and coarse cytoplasmic granulations; immunophenotype is characteristic in most cases. However, definitive laboratory diagnosis should be performed by detecting t(15;17) or by *PML-RAR α* fusion protein. **Objectives:** To compare cytomorphology, flow cytometry, and classical cytogenetic of bone marrow samples from patients with APL, treated at the Complexo Hospital de Clínicas da Universidade Federal do Paraná (CHC-UFPR), as well as describe the possible discrepancies between the methodologies. **Method:** Retrospective analysis of APL cases treated at the CHC-UFPR from January 2000 to July 2018. **Results:** Eighty-eight patients (42 man/46 woman; mean age: 34 years), 42.1% of them presented a high-risk prognosis. Flow cytometry was performed in 83 cases (94.3%); karyotype was performed in 79 cases (89.7%), but translocation t(15;17) was confirmed in only 53 cases (60.2%). From the 28 patients with a non-conclusive karyotype; fourteen (15.9%) of them presented the *PML-RAR α* transcript in the molecular analysis. In total, 35 patients (39.8%) performed research of the *PML-RAR α* gene by molecular biology. Only 45 patients (51.1%) presented concordant diagnosis among the three technical exams (morphology, flow cytometry and cytogenetics). Overall survival was 67% at 4.8 years, with 29 deaths. **Conclusion:** Genetic confirmation was observed in 76.1% of samples, 60.2% by conventional cytogenetics and 15.9% by molecular biology. There was a disagreement between the methodologies, and a low sensibility of the conventional cytogenetics, demonstrating the importance of performing molecular techniques for diagnostic confirmation.

Key words: acute promyelocytic leukemia; cytogenetics molecular biology; flow cytometry/cytology.

RESUMO

Introdução: A leucemia promielocítica aguda (LPA) é causada pela translocação t(15;17)(q24;q21), cujo produto é a oncoproteína de fusão *PML-RAR α* (leucemia promielocítica-receptor alfa do ácido retinoico). A morfologia dos promielócitos leucêmicos é habitualmente característica, com presença de faggot cells (células em maços ou feixes) e granulações citoplasmáticas grosseiras; o imunofenótipo é característico na maioria dos casos. Porém, o diagnóstico laboratorial definitivo deve ser feito pela detecção da t(15;17) ou pela oncoproteína *PML-RAR α* . **Objetivos:** Comparar a citomorfologia, a citometria de fluxo e a citogenética clássica de amostras de medula óssea de pacientes com LPA atendidos no Complexo Hospital de Clínicas da Universidade Federal do Paraná (CHC-UFPR), bem como descrever as possíveis discrepâncias entre as metodologias. **Método:** Análise retrospectiva dos casos de LPA atendidos no CHC-UFPR entre janeiro de 2000 e julho de 2018. **Resultados:** Dos 88 pacientes (42 homens e 46 mulheres; média de idade: 34 anos), 42,1% apresentaram prognóstico de alto risco. A citometria de fluxo foi realizada em 83 casos (94,3%); o cariótipo, em 79 casos (89,7%), mas a translocação foi confirmada em apenas 53 (60,2%) casos. Dos 28 pacientes com cariótipo não conclusivo, 14 (15,9%) tinham a presença do transcrito *PML-RAR α* . No total, 35 pacientes (39,8%) realizaram a pesquisa do gene *PML-RAR α* por biologia molecular. Quarenta e cinco pacientes (51,1%) obtiveram diagnóstico

concordante entre as metodologias (morfologia, citometria de fluxo e citogenética). A sobrevida global foi de 67% em 4,8 anos, com 29 óbitos. **Conclusão:** A confirmação genética foi realizada em 76,1% das amostras, sendo 60,2% por citogenética e 15,9% por biologia molecular. Houve discordância entre as metodologias e baixa sensibilidade da citogenética convencional, o que demonstra a importância da realização de técnicas moleculares para confirmação diagnóstica.

Unitermos: leucemia promielocítica aguda; citogenética; biologia molecular; citometria de fluxo/citologia.

RESUMEN

Introducción: La leucemia promielocítica aguda (LPA) es causada por la translocación $t(15;17)(q24;q21)$, cuyo producto es la oncoproteína de fusión PML-RAR α (proteína de la leucemia promielocítica-receptor alfa de ácido retinoico). La morfología de los promielocitos leucémicos suele ser típica, con presencia de células faggot (células en baces) y gruesas granulaciones citoplásmicas; el inmunofenotipo es característico en la mayor parte de los casos. No obstante, el diagnóstico final de laboratorio debe ser hecho por la detección de la $t(15;17)$ o por la oncoproteína PML-RAR α . **Objetivos:** Comparar la citomorfología, la citometría de flujo y la citogenética clásica de muestras de médula ósea de pacientes con LPA asistidos en el Complejo Hospital de Clínicas da Universidade Federal do Paraná (CHC-UFPR), así como describir las posibles discrepancias entre los métodos. **Método:** Análisis retrospectivo de los casos de LPA asistidos en el CHC-UFPR entre enero de 2000 y julio de 2018. **Resultados:** De los 88 pacientes (42 hombres y 46 mujeres; edad promedio: 34 años), 42,1% presentaron pronóstico de alto riesgo. Citometría de flujo se realizó en 83 casos (94,3%); cariotipo en 79 casos (89,7%), pero la translocación se confirmó en sólo 53 (60,2%) casos. Entre los 28 pacientes con cariotipo no concluyente, 14 (15,9%) presentaron el transcrito PML-RAR α . En total, 35 pacientes (39,8%) realizaron la pesquisa del gen PML-RAR α por biología molecular. Cuarenta y cinco pacientes (51,1%) tuvieron diagnóstico acorde entre los métodos (morfología, citometría de flujo y citogenética). La supervivencia global fue de 67% en 4,8 años, con 29 muertes. **Conclusión:** Hubo confirmación genética en 76,1% de las muestras, siendo 60,2% por citogenética y 15,9% por biología celular. Hubo desacuerdo entre los métodos y baja sensibilidad de la citogenética convencional, lo que demuestra la importancia de la realización de técnicas moleculares para confirmación diagnóstica.

Palabras clave: leucemia promielocítica aguda; citogenética; biología molecular; citometría de flujo/citología.

INTRODUCTION

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML), first described in 1957 by Hillestad, with unique clinical, morphological and molecular characteristics⁽¹⁾. The APL is characterized by the presence of the translocation $t(15;17)(q22; q21)$ ⁽²⁾, which results in the breakage and fusion of the promyelocytic leukemia (PML) gene, located on chromosome 15, with retinoic acid receptor alpha (RAR α) located on chromosome 17. The final product of the translocation is the fusion of the genes encoding the hybrid proteins PML-RAR α and RAR α -PML⁽³⁾, oncoproteins with sensitivity to retinoid action⁽⁴⁾. This oncoprotein causes interruption of maturation and the accumulation of cells in the stage of abnormal promyelocytes in the bone marrow⁽⁵⁾.

The presence of leukemic promyelocytes is occasionally observed in the peripheral blood; however, bone marrow

analysis is essential for diagnosis, especially in cases with severe pancytopenia⁽⁶⁾. APL may present as typical morphology, with hypergranular promyelocytes, or as the microgranular form. In the hypergranular form, the size and shape of the nucleus are irregular, and the cytoplasm is marked by several dense granules, grouped in the form of Auer rods, which characterizes the faggot cells. In the hypogranular variant, the nucleus is bilobed and the cytoplasm presents few or no granule⁽⁷⁾.

Although a morphological diagnosis is highly suggestive of APL, this is not enough to characterize it, and performing complementary exams are necessary, such as cytochemistry, multiparametric flow cytometry (MFC), conventional cytogenetic and molecular techniques. In the cytochemical analysis, neoplastic promyelocytes stain strongly with the myeloperoxidase or Sudan black reactions⁽⁸⁾. The immunophenotype study by MFC evidences the presence of blasts with high autofluorescence, which expresses myeloid markers such as CD117, CD13 and CD33, the

latter with homogeneous pattern of fluorescence intensity, and where the CD34 hematopoietic precursor cell marker and human leukocyte antigen (HLA)-DR are usually negative. Unlike normal promyelocytes, markers indicative of myelogenous maturity CD11b and CD15 are negative or of very low expression. Because it is a quick method, MFC reinforces the diagnostic suspicion of APL and helps in early therapeutic indication, but it is not an adequate method for the definitive diagnosis, which should be performed by cytogenetic and molecular techniques⁽⁹⁾. It is important to emphasize that the three methodologies are not exclusive, but complementary for the accurate diagnosis of APL.

Although all patients with APL present t(15;17) or a variant of this translocation, these translocations are detected in only 70%-96% of patients at diagnosis using conventional cytogenetic methods. Rare cases of APL lacking the classic t(15;17)(q21.1;q21.2) on routine cytogenetic studies have been described with complex variant translocations, additional chromosome or with the submicroscopic insertion of *RARα* into *PML* leading to the expression of *PML-RARα* transcript⁽¹⁰⁾.

OBJECTIVE

This study aims to compare the results obtained in bone marrow morphological analysis, immunophenotype by flow cytometry and conventional cytogenetics from APL patients treated at Complexo Hospital de Clínicas da Universidade Federal do Paraná (CHC-UFPR).

METHODOLOGY

Retrospective analysis of patients with a suspected morphological diagnosis of APL treated at CHC-UFPR between January 2000 and July 2018.

The bone marrow morphology, classic cytogenetic and MFC results files were consulted and data were confronted, seeking to analyze the agreement between the three methodologies.

MFC was performed in BD FACS Calibur™ using four-color panels, staining with the following combinations of fluorochrome-conjugated monoclonal antibodies [fluorescein isothiocyanate (FITC), phycoerythrin (PE) and either peridinin-chlorophyll-protein (PerCP) or the PE-cyanine 5 (PE-Cy5), fluorochrome tandem, and allophycocyanin (APC)] directed against surface antigens: CD11b, CD13, CD14, CD15, CD19, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, CD123, HLA-DR. In addition, the expression of MPO,

CD79a, and CD3 was also explored at the cytoplasmic level. After the 2017 year, FACS Canto II™ was introduced and eight-color Euroflow panel was performed in all the leukemia cases.

Conventional cytogenetic was performed as broadly described by Gus (2011)⁽¹¹⁾. Molecular analysis of *PML-RARα* transcript began to be performed systematically after the year 2006 when the site entered to the corporate Brazilian study named International Consortium on Acute Promyelocytic Leukemia (IC-APL)⁽¹²⁾. After this year all patients with molecular analysis performed were considered for confirmation of the disease.

Patient selection

Initially, 95 patients with a suspected cytomorphologic diagnosis for APL were selected between January 2000 and July 2018.

Four patients who did not undergo cytogenetics and flow cytometry tests for comparison were excluded. After morphological and flow cytometry review, two other patients who were diagnosed with AML with myelodysplasia-related changes, and one which was confirmed as acute monoblastic leukemia NOS, were also excluded. Cytogenetic profiles of these patients are described in **Table 1**.

To calculate the sensibility and specificity of classic cytogenetic analysis, we exclude the nine cases where the exam was not performed and consider the 82 remained cases (53 true-positive cases, 26 false-negative cases, and three true-negative cases). There were no false-positive cases in this cohort.

TABLE 1 – Karyotype and diagnosis of the three patients excluded from the study

Patient	Karyotype	Final diagnosis
1	(39~46,XY,del(7)(q22),del(9)(q21),add(19)(p13)[cp16]/idem,Y,-22 [03])	AML with myelodysplasia-related changes
2	46,XY,de(11)(q23)[2]/46,XY[6]	AML with myelodysplasia-related changes
3	46,XY,del(7)(q32)[3]/47,XY,sl,+8[31]	Acute monoblastic leukemia NOS

AML: acute myeloid leukemia; NOS: not otherwise specified.

RESULTS

A total of 88 patients were evaluated, 42 men and 46 women, with a median age of 34 years (ranging from 12 to 78 years).

Only 66% of the cases could be classified according to the prognostic risk due to the availability of the data. The platelets median in this period were $31 \times 10^9/l$ (range $7 \times 10^9/l$ to $528 \times 10^9/l$),

and leukocytes $5.48 \times 10^9/l$ (range $63 \times 10^9/l$ to $122.92 \times 10^9/l$). As a result, eleven patients (19.3%) were classified as low risk, twenty-two (38.6%) patients as intermediate risk, and 24 (42.1%) as high risk.

Bone marrow or peripheral blood morphological analyses were considered the gold standard of the study. Only two cases without bone marrow analysis were included, both of which exhibited peripheral blood morphology highly suggestive of APL. Ten cases of microgranular or variant morphology (dumbbell nuclei and few cytoplasm granules) were seen. Myeloperoxidase (MPO) cytochemistry analysis was positive in all of them.

From the 88 patients with a suggestive morphological analysis of APL, 83 presented MFC examinations (94.3%) (**Figure 1**).

The conventional cytogenetic analysis was performed in 79 cases (89% of the sample), but the translocation (15; 17) was confirmed in 53 cases (60.2%). Thirteen patients presented a normal karyotype and five presented no metaphases for evaluation. Nine patients did not perform the karyotype, seven of them before the entrance in International Consortium (IC)-APL study. These data are shown in **Figure 2**.

As expected, the specificity of classic cytogenetic was 100% in the morphologic suspected cases, but the sensibility was 67% (53 true-positive and 26 false-negative cases) when the not performed cases were considered.

The search of *PML-RAR α* transcript by molecular biology was performed by the IC-APL study, since December 2005, in 35 patients (39.8%), all of them were positive. In regard to the 26 patients with non-conclusive karyotype, 14 (53.8% of tested, 15.9% of total cases) presented confirmation of the *PML-RAR α* transcript presence by molecular analysis.

In total, 35 patients (39.8%) had *PML-RAR α* gene research performed by molecular biology in samples sent to Ribeirão Preto, to the IC-APL study.

Before 2006, we found seven cases that did not have cytogenetic analysis performed, four cases without metaphases and five cases with normal karyotype. After this period only two patients did not perform the karyotype and one case did not show growth of metaphases in cell culture.

From the patients with translocation t(15;17) confirmed, there were additional cytogenetic alterations in six patients, two of which were considered complex karyotypes. There were eight patients who presented cytogenetic changes: +8 ($n = 2$), del11q ($n = 1$), del17p ($n = 2$) and marker chromosomes ($n = 1$), but did not present translocation t(15;17).

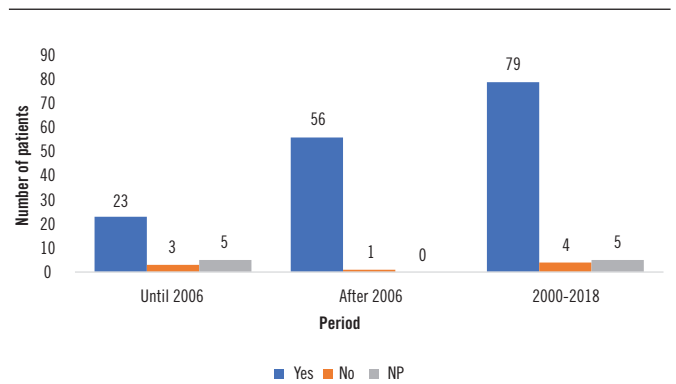


FIGURE 1 – MFC tests suggestive of APL (yes, no or not performed), before and after 2006, and in the total study period

MFC: multiparametric flow cytometry; APL: acute promyelocytic leukemia; NP: not performed.

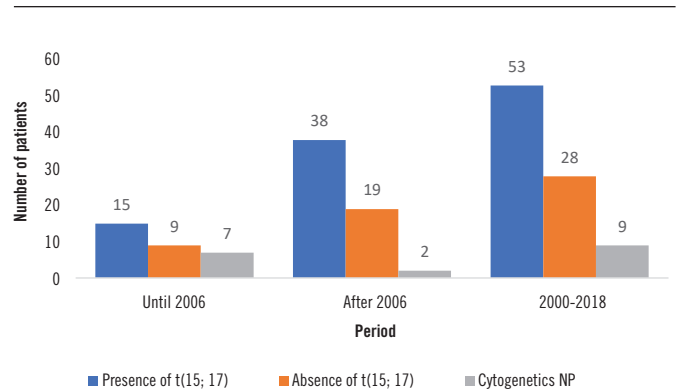


FIGURE 2 – Conventional cytogenetics of patients with translocation t(15; 17) present, absent or unrecognized before and after 2006, and in the total study period

NP: not performed.

One case in this sample presented the variant translocation t(11; 17)(q13;q21), and one case presented a triple translocation t(5;15;17)(q13;q24;q21).

A translocation involving chromosome 15 and 17 or its variants were detected by conventional cytogenetic in 60.2% of cases, and confirmed by molecular analysis in 15.9%, totaling 76.1% of samples confirmed by genetic methods in this cohort. These results are shown in **Table 2**.

There were five cases of disagreement between morphology, flow cytometry and conventional cytogenetic and 32 cases of agreement only between immunophenotype and morphology, with normal karyotype in 13 cases and absence of metaphases in five cases. Nine did not perform culture and six performed another karyotype.

TABLE 2 – Karyotype and *PML-RAR α* fusion results

Patient	Karyotype	<i>PML-RARα</i> fusion
1, 5, 7, 12, 13, 14, 17, 34, and 86	Not performed	NP
9, 15, 19, and 29	No metaphases	NP
47	No metaphases	Yes
25	Normal karyotype 46,XY[20]	NP
26	Normal karyotype 46,XX[15]	NP
31	Normal karyotype 46,XX[9]	Yes
33	Normal karyotype 46,XX[20]	NP
36	Normal karyotype 46,XY[5]	Yes
40	Normal karyotype 46,XY[20]	NP
41	Normal karyotype 46,XX[21]	NP
60	Normal karyotype 46,XX[20]	Yes
63	Normal karyotype 46,XY[20]	NP
68	Normal karyotype 46,XX[11]	Yes
75	Normal karyotype 46,XX[3]	Yes
78	Normal karyotype 46,XX[13]	Yes
84	Normal karyotype 46,XY[20]	Yes
2	46,XY,t(15;17)(q24;q21)[02]	NP
3	46,XY,t(15;17)(q24;q11)[19]/46,XY[1]	NP
4	46,XX,t(15;17)(q24;q11)[10]	NP
6	46,XX,t(15;17)(q24;q21)[13]	NP
8	46,XY,t(15;17)(q24;q21)[19]/46,XY[01]	NP
10	46,XY,t(15;17)(q24;q21)[11]/46,XY[5]	NP
11	46,XX,t(15;17)(q24;q21)[11]	NP
16	46,XY,t(15;17)(q24;q11)[6]/46,XY[14]	NP
18	46,XY,t(15;17)(q24;q11)[4]/46,XY[08]	NP
20	46,XY,t(15;17)(q24;q11)[12]/46,XY[5]	NP
21	46,XX,t(15;17)(q24;q21)[1]/46,XX[1]	NP
22	46,XX,t(15;17)(q24;q11)[3]/46,XX[20]	NP
23	46,XX,t(15;17)(q24;q11)[15]/46,XX[5]	NP
24	46,XX,t(15;17)(q24;q21)[2]/46,idem,del(1)(p22)[6]/46,XX[2]	NP
27	46,XX,t(15;17)(q24;q21)[14]/46,XX[6]	NP
32	46,XY,t(15;17)(q24;q11)[11]/46,XY[9]	NP
35	46,XX,t(15;17)(q24;q21)[7]/46,XX[13]	NP
37	46,XX,t(15;17)(q24;q11-21)[5]/46,XX[11]	Yes
39	46,XX,t(15;17)(q24;q21)[18]/46,XX[2]	NP
42	46,XX,t(15;17)(q24;q21)[11]/46,XX[10]	NP
43	46,XY,t(15;17)(q24;q21)[5]/46,XY[9]	Yes
44	46,XX,t(15;17)(q24;q11)[14]/46,XX[6]	Yes
45	46,XY,t(15;17)(q24;q21)[15]/46,XY[5]	Yes
50	46,XX,t(15;17)(q24;q21)[7]/46,XX[13]	Yes
51	46,XX,t(15;17)(q24;q21)[20]	Yes
52	46,XX,t(15;17)(q24;q21)[5]/46,XX[21]	NP
55	46,XY[11]/46,XY,t(15;17)[2]	NP
56	46,XY,t(15;17)(q24;q21)[4]/46,XY[16]	NP
57	46,XY,t(15;17)(q24;q21)[12]/46,XY[8]	NP
62	46,XY,t(15;17)(q24;q21)[13]/46,XY[2]	Yes
64	46,XY,t(15;17)(q24;q21)[15]/46,XY[5]	Yes
65	46,XX,t(15;17)(q24;q21)[13]/46,XX[4]	NP
66	46,XX,t(15;17)(q24;q21)[13]/46,XX[1]	Yes
70	46,XY,t(15;17)(q24;q21)[15]	Yes
72	46,XX,t(15;17)(q24;q21)[5]/46,XX[23]	NP
74	46,XY,t(15;17)(q24;q21)[18]/46,XY[6]	NP
76	46,XY,t(15;17)(q24;q21)[04]/46,XY[03]	
79	46,XY,t(15;17)(q24)(q21)[5]	NP
80	46,XY,t(15;17)(q24)(q21)[8]/46,XY[11]	Yes
81	46,XY,t(15;17)(q24;q21)[11]/46,XY[4]	Yes

82	46,XX,t(15;17)(q24;q21)[17]	Yes
83	46,XY,t(15;17)(q24;q21)[4]/46,XY[9]	Yes
85	46,XY,t(15;17)(q24;q21)[9]/46,XY[20]	NP
87	46,XX,t(15;17)(q24;q21)[13]/46,XX[2]	Yes
88	46,XX,t(15;17)(q24;q21)[19]/46,XY[1]	NP
48	46,XX,t(5;11)(q31;q23);t(15;17)(q24;q21)[8]/46,XX[7]	Yes
49	46,XY,inv(9)(p12q13),t(15;17)(q24;q21)[20]	Yes
54	46,XX,t(15;17)(q24;q21), add(16)(p?) [20]	NP
67	46,XX,t(15;17)(q24;q21)[9]/46,sl,del(11)(q23)[2]/46,XX[9]	Yes
46	46,XY,t(15;17)(q24;q21)[6]/47,sl,+8[4]/47,sdl,inv(9)(p11q13)[7]/46,XY[6]	Yes
77	46-47,X,-X[11],?t(15;17)(q24;q21)[4],+mar[18],+marX2[3][cp26]/46,XX[2]	Yes
71	46,X,?del(X)(q21),?t(5;15;17)(q13;q24;q21),?del(18)(p10)[14]	NP
58	44~46,XX,?,+8,t(11;17)(q13;q21),-14,del(22)(q11)[9]/46,XX[12]	
28	47,XX,-6,+mar,+21[03]/46,XX,-6,+mar[14]	NP
30	46,XX, del(17)(p11) [11] / 46,XX [16]	NP
38	46,XX,-E,+mar [7] / 46,XX [14]	
53	47,XY,+8[9]/46,XY[21]	
59	46,XY,del(11)(q23)[2]/46,XY[18]	Yes
61	Complex karyotype 7 and 11 [22]/46,XX[2]	Yes
69	47,XY?+8[6]/46,XY[16]	Yes
73	46,XY,?del(17)(q24)[5]/46,XY[13]	Yes

PML-RAR α : promyelocytic leukemia-retinoic acid receptor alpha; NP: not performed; Yes: positive.

Only 45 patients (51.1%) agreed on the diagnosis among the three exams (morphology, flow cytometry and conventional cytogenetic) (**Figure 3**).

Treatment and follow-up

Most patients received all-trans retinoic acid (ATRA) therapy (45 mg/m² orally, until complete remission), which was initiated as soon as possible after the presumptive diagnosis of APL, based on morphological characteristics,

immunophenotype, and clinical judgment. Between 2001 and 2006, the treatment protocol was based on the combination of ATRA with chemotherapy (daunorubicin 60 mg/m² and mitoxantrone 10 mg/m²), followed by consolidation with bone marrow autologous transplantation. After 2006, the induction protocol was replaced by anthracycline on days 2, 4, 6 and 8, associated with the ATRA, according to the treatment algorithm proposed by the IC-APL in 2006⁽¹²⁾.

At a median segment of 4.83 years, 59 patients are alive with complete response (overall survival of 67%). There were 18 early deaths (up to three months of therapy) and 11 late deaths, of which six deaths due to confirmed relapse. Fourteen from the 29 deaths reported were of patients at high risk (48.3%).

DISCUSSION

APL is a type of leukemia caused by the translocation t(15; 17)(q24;q21), whose product is the *PML-RAR α* oncoprotein. The initial clinical-morphological evaluation of APL is essential for the early initiation of therapy; however, it is known that this is not always conclusive⁽¹³⁾.

APL requires special attention among AML subtypes for its prognostic and therapeutic implications. With current therapy, 70%-80% of patients remain disease-free survival for at least five

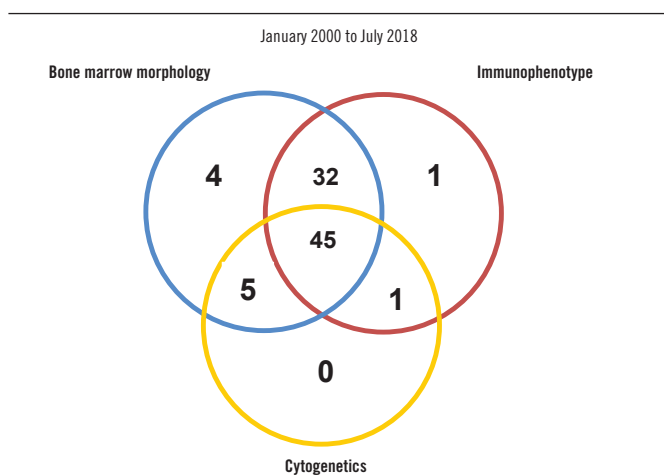


FIGURE 3 – Set diagrams related to bone marrow morphology, immunophenotype and cytogenetics for patients diagnosed from January 2000 to July 2018

years⁽¹⁴⁾. It is known that patients with *PML-RARα* fusion protein present coagulopathy, 40% of whom develop severe bleeding, such as pulmonary and cerebral, which may be lethal⁽¹⁵⁾. Due to this complication, APL was for many years considered to be one of the most fatal subtypes of AML. However, since the introduction of ATRA as a treatment, it has become the most curable type of AML⁽¹⁶⁾.

The definitive APL diagnosis should be confirmed by techniques capable of detecting the translocation t(15;17) (q24;q21) or the *PML-RARα* rearrangement, or alterations involving the *RARα* gene. Conventional cytogenetics, fluorescence *in situ* hybridization (FISH), and reverse transcriptase-polymerase chain reaction (RT-PCR) are the available options. The FISH technique has lower sensitivity than RT-PCR, but is more specific.

The conventional karyotype presents the advantage of allowing the diagnosis of additional cytogenetic alterations, however, the execution time is longer and the number and quality of metaphases may be variable⁽⁹⁾. Rare cases of APL lacking the classic t(15;17) (q24;q21) on routine cytogenetic studies have been described with complex variant translocations, additional chromosome or with the submicroscopic insertion of *RARα* into *PML* leading to the expression of *PML-RARα* transcript⁽¹⁰⁾. Despite the limitations of the technique, cytogenetics seems to have good specificity and positive predictive value in this pathology.

In the present study, the t(15;17) translocation or its variants were confirmed by conventional cytogenetic analysis in only 60.2% of the sample (53 cases), and 14 (15.9%) additional patients with inconclusive karyotype could be confirmed by the presence of the *PML-RARα* transcript by molecular analysis. In total 76.2% of samples from this cohort were confirmed by genetic methods. This result is similar to that obtained by Berger and Coniat (2000)⁽¹⁰⁾, where 62.8% of the 121 studied patients presented this cytogenetic alteration. As expected, the specificity of classic cytogenetic was 100% in this study, but the sensibility was quite low (67%). According to literature, the translocation t(15;17) is present in up to 90% of the cases in some studies⁽¹⁷⁾. The low percentage of translocation detection in conventional cytogenetic may be due to the limitation of the technique since the analysis can be performed on cells that do not belong to the neoplastic clone (normal clone), there may be difficulties in identifying the translocation or even due to the presence of cryptic rearrangements that mask the translocation^(14, 17).

In this study, in five cases, there was no success in obtaining metaphase, four of them occurred before 2006. Besides this, the

large number of non-realized tests (seven cases before 2006 and two cases after this date) can be explained by the absence of a rigid protocol for evaluation and follow-up of patients up to this period. The association with IC-APL⁽¹²⁾, established at the end of 2005, could contribute to the improvement of diagnostic tools and to the better prognosis of APL in our institution, since a standardized approach was implemented for a specific and immediate diagnosis, in addition to changes in treatment, measures of support and follow-up of the cases. It was possible to observe an increase in the conclusive diagnosis from the year 2006 when the IC-APL protocol was introduced in our site. There was a significant improvement in the correct diagnosis of this disease due to the establishment of the correct flow of the diagnostic tests to be performed, resulting in a decrease in the number of exams that were not processed and in an increase in the conclusive diagnosis, mainly due to the implantation of the *PML-RARα* transcript by molecular biology.

Approximately 30% to 40% of patients with APL may have additional cytogenetic alterations beyond t(15;17). The most frequent are chromosomes 8 and 21 trisomies, structural abnormalities on chromosome 9 and isochromosome 17q⁽⁸⁾. In this study, it was found two cases with trisomy of chromosome 8 and one case with a deletion in the long arm of chromosome 17.

Besides this, in about 2% of cases the *RARα* gene can be fused with a gene other than *PML*, and promyelocytic leukemia zinc finger (PLZF) is a result of t(11;17)(q23;q21), NPM (nucleophosmin), resulting from t(5;17)(q35;q21), and nuclear mitotic apparatus (NuMA), resulting from t(11;17)(q13;q21)⁽¹⁸⁾. Complex translocations with more than two chromosomes can also be found, with 33 proved cases reported in the literature since the year of 2009⁽¹⁹⁾. In this study, we found two variant translocations: translocation t(11;17)(q13;q21) in one case and t(5;15;17)(q13;q24;q21) in the other.

Flow cytometric immunophenotyping has been useful in distinguishing APL from the other types of AML, since the absence of expression of the HLA-DR and CD34 markers are characteristic but not specific for APL⁽²⁰⁾. AML with *FLT3* and *NPM1* mutations are examples of other diseases that may also present negativity for these markers⁽²¹⁾. This differentiation is quite important because this technique can help to guide disease-specific therapy, increasing the APL patient's survival rate. In our study, there were three patients whose integrated diagnosis between morphology, MFC and cytogenetic were essential for the exclusion of APL.

The risk classification analysis was performed based on Cingam and Koshy study (2017)⁽²²⁾. Most patients in this study were classified as high-risk (41.1%), or intermediate-risk (39.3%), or low-risk (19.3%). This result was different from that found in European centers, which have a higher prevalence of intermediate-risk patients (55.5%), followed by low-risk patients (37.8%) and lastly, high-risk patients (16.7%)⁽²³⁾. The overall survival was 67% in five years, but almost half of the 29 deaths reported occurred in patients at high risk, indicating the importance of this classification in the prognostic evaluation of the disease.

CONCLUSION

A translocation involving chromosome (15;17)(q24;q21) or its variants was detected in patients suspected of having APL in 60.2% of cases by conventional cytogenetics and in 15.9% additional cases by molecular analysis, totaling 76.1% of samples confirmed by genetic methods in this cohort.

The low sensibility of conventional karyotype in APL demonstrated the importance of performing molecular techniques for diagnostic confirmation.

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