



Revista Brasileira de Hematologia e Hemoterapia Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org



Special article

Guidelines on the diagnosis and treatment for acute promyelocytic leukemia: Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular Guidelines Project: Associação Médica Brasileira - 2013

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Acceptance date

22 November 2013

Introduction

The guidelines project is a joint initiative of the Associação Médica Brasileira and the Conselho Federal de Medicina. It aims to collect information to standardize decisions and help create strategies during diagnosis and treatment. These data were prepared and are recommended by the Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular (ABHH). Even so, all possible decisions should be evaluated by the physician responsible for diagnosis and treatment according to the patient's setting and clinical status.

Description of the evidence collection method

The members of the ABHH Committee responsible for writing the diagnostic and treatment guidelines for acute promyelocytic leukemia (APL) prepared the main questions related to clinical diagnosis and treatment. Eighteen questions were structured using the Patient/Problem, Intervention, Comparison and Outcome (PICO) system. The search strategy, with specific clinical questions (Appendix 1), was applied to the key scientific databases (MEDLINE PubMed, Embase, SciELO, Lilacs and Cochrane Library). The retrieved articles were submitted to a critical appraisal and categorized according to the strength of evidence, giving support to elaborate the answers to the questions. Each selected reference was classified according to the degree of recommendation using the

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DOI: 10.5581/1516-8484.20140018

Oxford Classification. Each recommendation was discussed by the committee and a consensus attained. The development of these recommendations was completely supervised by experts on evidence-based guidelines.

Recommendation degree and evidence level

A: Experimental or observational studies of better consistency

B: Experimental or observational studies with less consistency

C: Case reports (uncontrolled studies)

D: Opinion without critical evaluation based on consensus, physiological studies or animal models

Aims

To define parameters for clinical diagnosis, assess the severity and standardize steps and options for treatment, maintenance and monitoring of patients with acute promyelocytic leukemia (APL).

Which methods are used to confirm the diagnosis of acute promyelocytic leukemia?

P – Acute promyelocytic leukemia

I – Karyotyping, cytogenetics, flow cytometry, fluorescence in situ hybridization, reverse transcriptase polymerase chain reaction or bone marrow cell morphology

O – Diagnosis

The classic form of APL, according to World Health Organization (WHO) criteria, presents a characteristic translocation, $t(15;17)(q22;q12)$, the PML-RAR α fusion transcript (D).¹ However, variant chromosomal aberrations are identified in up to 5% of APL cases including, $t(11;17)(q23;q21)$, $t(5;17)(q35;q12-21)$, $t(11;17)(q13;q21)$ and *der(17)*, in which the *retinoic acid receptor alpha* (RAR α) gene is fused to the PLZF, NPM, NuMA and STAT5b genes, respectively. PLZF-RAR α , in general, presents atypical morphology compared to the classic PML-RAR α fusion transcript, with regular nuclei and hypogranular or microgranular cells, abundant cytoplasm, blasts with more condensed chromatin, absence of Auer rods and an increase in the number of pseudo-Pelger-Huet cells. The different variants of APL, although rare, are challenging for early diagnosis, which is achieved with the combination of morphological analysis, clinical presentation and genetic testing² (C). There are rarer situations in which rearrangements of the RAR α gene are masked or complex; these are only characterized by G-banded karyotyping, with fluorescence in situ hybridization (FISH) and/or polymerase chain reaction (PCR) required for confirmation. Furthermore, in 20 to 40% of cases of APL, in addition to the $t(15;17)$ mutation, other clonal changes are observed at diagnosis such as trisomy 8; the importance of these changes continue to be controversial (A).³

One antibody was investigated as a possible marker to diagnose APL in patients with a mean age of 35 years (range:

9-75 years) who had the PML-RAR α fusion transcript (standard diagnosis) identified by reverse transcriptase polymerase chain reaction (RT-PCR). In this work, the diagnostic efficiency of RT-PCR was compared with morphology, cytochemistry and immunofluorescence using the PG-M3 monoclonal antibody; the time to perform this test is two hours. Morphology alone enabled the correct diagnosis in 88.9% of cases with 11.1% of false-negative results [sensitivity: 88.9%; specificity: 57.1%; positive predictive value (PPV): 72.7%; negative predictive value (NPV): 80%; verisimilitude ratio (VR): 2.07]; the association of morphology with cytochemistry led to a 91.7% diagnosis rate with 8.3% of false negatives (sensitivity: 91.7%; specificity: 78.6%; PPV: 84.6%; NPV: 88%; VR: 4.28). With immunofluorescence, the diagnosis was correct in 94.4% of the cases with 5.6% of false negatives (sensitivity: 94.4%; specificity: 92.9%; PPV: 94.4%; NPV: 92.9%), resulting in a statistically significant difference in respect to the morphological evaluation in isolation (p -value < 0.01) (B).⁴

In a study with 349 patients with ages ranging from 1 to 81 years (median: 46.2 years) not including patients with the variants of APL, the reaction with the anti-PML antibody was positive in 98.9% of cases when the test was performed using bone marrow aspirate and 96.9% when peripheral blood was used; conventional cytogenetic demonstrated the $t(15;17)(q24;q21)$ in 91% patients and RT-PCR and FISH were positive for the PML-RAR α rearrangement in 98% and 96% of the cases, respectively. The results indicate that the immunofluorescence reaction using the anti-PML antibody can be used as a fast and reliable first-line test to confirm when there is clinical or morphological suspicion of APL in order to expedite the start of treatment and prevent fatal hemorrhagic complications (B).⁵

FISH was employed to detect the PML-RAR α rearrangement in APL even though it was already well known that the sensitivity of this technique is insufficient to detect low levels of the disease. Eighty-two bone marrow samples were studied including samples from 30 healthy bone marrow donors, 33 patients with untreated APL, 14 patients with treated APL and five with variant translocations. In this study, using dual-color, dual fusion fluorescence in situ hybridization (D-FISH) to detect the PML-RAR α fusion transcript and RAR α gene abnormalities, the sensitivity was 98% and the specificity was 100%. If the patient has a $t(15;17)$, this method has an excellent analytical sensitivity and can detect disease if more than 0.6% of 500 nuclei are marked. It also detects alternative translocations involving the RAR α gene in more than 1.6% of 500 nuclei but not those of the PML gene. Thus, it is suggested that interphase FISH may be as good as conventional cytogenetics to investigate patients with APL. For patients with a possible diagnosis of APL, D-FISH would initially be indicated because of the speed of the test. When the results of FISH are normal, conventional cytogenetics would complement the investigation by detecting chromosomal abnormalities associated with leukemias other than APL or by identifying variants. According to these authors, except for the $t(15;17)$ mutation, other cytogenetic changes have no diagnostic or prognostic significance in APL, making conventional cytogenetics unnecessary when the PML-RAR α fusion transcript is detected by FISH (A).⁶

Eighty-five patients diagnosed with acute myeloid leukemia (AML), including 15 cases of APL, were studied to evaluate the

diagnosis of APL using the anti-PML monoclonal antibody, 5E10. There was agreement in respect to the detection of the t (15; 17) by immune fluorescence and RT-PCR in 14 out of 15 cases (93.3%) without false positive results. The discordant case was a patient with a 5' breakpoint in the PML gene that did not express the reciprocal t (15; 17) fusion product. According to these authors, in addition to the traditionally described techniques used for the diagnosis of APL including morphology, immunophenotyping, conventional cytogenetics, FISH and RT-PCR, immunofluorescence with anti-PML monoclonal antibodies (5E10) allows the diagnosis of APL in 93.3% of cases, similar to the rate attained by RT-PCR but with the advantage of being faster (B).⁷

The recommendation of a panel of experts of the European Leukemia Net includes genetic confirmation of the diagnosis in leukemic cells in bone marrow aspirate. In addition to conventional karyotyping, FISH and RT-PCR, immunofluorescence with anti-PML antibodies can be performed. Diagnosis should be confirmed by molecular detection of the PML-RAR α fusion transcript or its molecular variants (D).⁸

A publication on APL treatment suggested that several techniques can be used to confirm diagnosis. G-banded karyotyping has the advantage of being highly specific detecting typical translocations and variants. However, karyotyping is not only costly, but it is time-consuming and does not detect cryptic rearrangements. RT-PCR, the gold standard test for the PML-RAR α fusion transcript, may be performed when there is leukopenia, but it has the disadvantages of possible contamination and technical problems that can lead to false-positive and false-negative test results. FISH can also be performed, although this technique adds little to karyotyping, and RT-PCR. Immunofluorescence with anti-PML antibodies is fast and is positive in most patients with atypical breakpoints (D).⁹

Recommendations

The diagnosis of APL is based on an evaluation of the clinical presentation, morphology, immunophenotyping, karyotype, RT-PCR, FISH and immunofluorescence with anti-PML monoclonal antibodies. The redundancy of diagnostic tests is beneficial because of the broad and indistinct characterization of the disease. However with the impossibility of completing the full set of recommended tests, identification of the PML-RAR α rearrangement or its product together with the clinical presentation, morphology and immunophenotyping favors the diagnosis of APL, even though some variants pass unnoticed using this restricted approach.

Which laboratory tests are used to evaluate coagulopathies in acute promyelocytic leukemia?

P – Acute promyelocytic leukemia

I – Coagulogram, D-Dimer, fibrinogen, platelets, prothrombin time, activated partial thromboplastin time, fibrin degradation products

O – Bleeding

The laboratory tests to predict bleeding in patients with APL are important to guide transfusion therapy. Among

the parameters analyzed are: percentage of leukemic cells, leukocyte and platelet counts at diagnosis, prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen levels. Bleeding was observed in patients presenting leukocytosis ($26.73 \pm 6.18 \times 10^9/L$ vs. $13.03 \pm 3.03 \times 10^9/L$; p -value = 0.026), prolonged PT ($4.85 \pm 0.70s$ vs. $2.59 \pm 0.28s$; p -value = 0.002) and prolonged aPTT ($3.98 \pm 1.68s$ vs. $0.96 \pm 0.93s$; p -value = 0.017). The remaining parameters showed no significant differences. When the PT was $\geq 5s$, there was a relative risk of bleeding of 6.14 with this being the most accurate parameter to predict a coagulopathy (B).¹⁰

One study investigated patients diagnosed with APL from December 1998 to March 2009 who initiated treatment with all-trans retinoic acid (ATRA - 45 mg/m²/day) combined with idarubicin [12 mg/m²/day on Day (D)1-D4] or cytarabine (100 mg/m² b.i.d. on D1-4) and daunorubicin (45 mg/m²/day D1-3) who were monitored daily to measure PT, aPTT, and lactate dehydrogenase (LDH), plasma fibrinogen and D-dimer serum concentrations (B).¹¹ There was no significant difference between groups with or without bleeding for LDH (p -value = 0.003), PT (p -value = 0.037), serum fibrinogen (p -value = 0.004) and D-dimer (p -value = 0.001) (B).¹¹

In an analysis of 279 patients with APL aged from 15 to 70 years, 6.5% had severe bleeding with the following risk factors being identified as pre-treatment predictors for bleeding: baseline fibrinogen concentration < 1 g/dL [ODDs ratio (OR): 3.28; 95% confidence interval (95% CI): 1.17-9.19; p -value = 0.024], white blood cell (WBC) count $20 \times 10^9/L$ (OR: 3.61; 95% CI: 1.14-11.4; p -value = 0.029) and the performance status between 2 and 3 (OR: 3.04; 95% CI: 1.02-9.02; p -value = 0.045) (B).¹²

Thirty-four patients with APL aged from 8 to 66 years, diagnosed by morphological criteria confirmed by the presence of the t (15;17) or PML-RAR α rearrangement and submitted to induction treatment with daunorubicin (50 mg/m²/day for four days) and ATRA [45 mg/m²/day until complete remission (CR)] were assessed for hemostatic abnormalities. The main features found were: abnormal platelet count in 91% of the cases (mean: $30 \times 10^9/L$; range: $3-191 \times 10^9/L$), reduced plasma fibrinogen concentration (≤ 100 mg/dL) in 61%, prolonged aPTT in 9% and prolonged PT in 44%. Episodes of severe bleedings occurred in 29% of the patients (B).¹³ There was a statistically significant difference in the multivariate analysis of hemostatic parameters comparing groups with or without severe bleeding only for the baseline WBC count which, when over $30 \times 10^9/L$, favored the development of severe bleeding (OR: 10; 95% CI: 1.0-10.5; p -value = 0.04) (B).¹³

Hemorrhagic complications were observed in 89.4% of 19 patients with APL with four deaths. The fatal hemorrhages were related to low levels of fibrinogen and low platelet counts. There was no significant difference in the baseline WBC count, the LDH concentration, PT and aPTT (C).¹⁴

Recommendations

WBC and platelet counts, PT and aPTT, and fibrinogen, LDH and D-Dimer concentrations should be investigated in the laboratory workup of coagulopathies in APL. However, low fibrinogen concentrations and prolonged PT are the parameters that best predict the likelihood of bleeding.

How can the risk of relapse in patients with acute promyelocytic leukemia be stratified?

P – Acute promyelocytic leukemia

I – Risk stratification

O – Recurrence

The prognostic factors that influence the risk of relapse in APL were evaluated in a series of 217 patients aged from one to 74 years, diagnosed with APL and confirmed by the presence of the PML-RAR α fusion transcript by the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA: n = 108) and the Spanish Programa para el Estudio de la Terapéutica en Hemopatía Maligna group (PETHEMA: n = 109). The variables analyzed were: age, gender, hemoglobin level, leukocyte count, platelet count, French-American-British (FAB) subtype (typical or variant) and the isoforms of the PML-RAR α fusion transcript (bcr-1, bcr-2 or bcr-3). In a univariate analysis, leukocytosis, thrombocytopenia and the classification of APL as typical or variant were significant for increased risk of relapse (p -value < 0.0001, p -value < 0.05 and p -value < 0.05, respectively). In multivariate regression analysis of relapse-free survival, the leukocyte and platelet counts were the only variables with independent prognostic value. The resulting predictive model for relapse-free survival allowed the classification of patients as low risk (leukocyte count $\leq 10 \times 10^9/L$, platelet count $> 40 \times 10^9/L$), intermediate risk (leukocyte count $\leq 10 \times 10^9/L$ and platelet count $\leq 40 \times 10^9/L$) and high risk (leukocyte count $> 10 \times 10^9/L$) with distinct curves of relapse-free survival (p -value < 0.0001) (B).¹⁵

In a retrospective study with 86 adults with newly diagnosed APL admitted to a Chinese medical service between April 2003 and June 2009, diagnosis was established according to the clinical and morphological criteria of the FAB classification and genetically confirmed by the presence of the t (15; 17)(q24; q21) by karyotyping and the PML-RAR α fusion transcript by RT-PCR. Patients were stratified into low risk when the total leukocyte count was $< 10 \times 10^9/L$ and platelet count $> 40 \times 10^9/L$, intermediate risk when the total leukocyte count was $< 10 \times 10^9/L$ and platelet count $< 40 \times 10^9/L$ and high risk when the total leukocyte count was $\geq 10 \times 10^9/L$. The mean follow-up was 37 months (range: 6-70 months) after induction and consolidation treatment when patients were in CR, the overall survival (OS) was 100%, 100% and 84.8%, respectively for each risk group, with a significant difference between high risk and intermediate/low risk groups (p -value = 0.0034), the event-free survival (EFS) rates were 100%, 94.4% and 89.7%, respectively and the cumulative incidence of relapse in the central nervous system (CNS) was 0%, 2.8% and 6.9%, respectively, with a significant difference between high risk and intermediate/low risk groups (p -value < 0.05) (B).¹⁶

In the evaluation of 36 patients with APL between 1990 and 2002 with a median age of 37 years, the risk stratification of patients was made on the basis of the criteria of the Italian GIMEMA and the Spanish PETHEMA studies (low risk when the leukocyte count $\leq 10 \times 10^9/L$ and the platelet count $> 40 \times 10^9/L$; intermediate risk when the leukocyte count was $\leq 10 \times 10^9/L$ and the platelet count $< 40 \times 10^9/L$ and high risk when the leukocyte count was $> 10 \times 10^9/L$). The OS at 32 months

was 66% and the disease free survival (DFS) at 42 months was 62% with a statistically significant difference between high-risk patients and the low/intermediate risk groups (p -value = 0.04). OS and DFS were not affected by age, variants of the PML-RAR α fusion transcript or additional karyotypic abnormalities. The DFS was not affected by gender, hemoglobin concentration, karyotype or isoforms of PML-RAR α fusion gene transcript (B).¹⁷

In 2008, the results of the long-term multicenter LPA99 study were published by the PETHEMA Group. Between November 1996 and June 2005, 792 patients were assessed, 732 of whom were eligible for the study. All patients had a diagnosis of APL and were inserted into the LPA96 and LPA99 protocols which encompassed 82 institutions in several countries. The accumulated incidence of relapse, DFS and OS at five years were 11%, 84% and 82%, respectively. At the end of the consolidation regimen, RT-PCR was performed to identify the PML-RAR α fusion transcript in 448 cases; three patients in the high-risk group remained PCR-positive. These data corroborate the findings of the LPA96 study (p -value = 0.028) in which five of 138 eligible patients (four of 44 in the high-risk group and one of 97 patients at intermediary risk) presented molecular persistence at the end of the consolidation regimen. Strategies adapted to the risk of each patient, prioritizing patients at high risk of relapse should be the focus of future studies (B).¹⁸

Recommendations

Stratification of patients with diagnosis of APL in respect to risk of relapse must include the leukocyte and platelet counts at the time of diagnosis. Patients are considered low risk when, at diagnosis, the leukocyte count $\leq 10 \times 10^9/L$ and the platelet count $> 40 \times 10^9/L$, intermediate risk when the leukocyte count $\leq 10 \times 10^9/L$ and the platelet count $\leq 40 \times 10^9/L$ and high risk when the leukocyte count $> 10 \times 10^9/L$.

How should coagulopathies in acute promyelocytic leukemia be treated?

P – Acute promyelocytic leukemia

I – Coagulopathy

O – Treatment

Thrombohemorrhagic syndromes remain the leading cause of death during induction therapy and of failure to obtain hematological remission in patients with APL. About 60 to 80% of patients have some clotting disorder at diagnosis. Currently, mortality rates secondary to thrombohemorrhagic events during induction occur in around 5 to 7% of patients eligible for clinical studies (B).^{14,19,20}

One study retrospectively evaluated 24 patients with morphologic diagnosis of APL. Historical comparisons of studies carried out from 1970 to 1976 compared chemotherapy with and without the use of prophylactic unfractionated heparin. Group I, consisting of seven patients with a median age of 47 years, received arabinosylcytosine and 6-thioguanine and presented CR in 14.3% of the cases with death occurring in 85.7% (57.1% died due to cerebral hemorrhage) during the induction treatment. Group II, consisting of eight patients

with a median age of 41 years, received arabinosylcytosine and daunorubicin and presented CR in 25% and death in 75% (62.5% by cerebral hemorrhage) during the initial treatment. Group III, consisting of nine patients with a median age of 35 years, received a chemotherapy scheme similar to group II associated with prophylactic heparin at doses varying from 5 to 20 U/kg/hour. This group presented CR in 77.8% and deaths in 22.2%, all due to cerebral hemorrhage. Thus, risk reduction was observed in 10.7% [number needed to treat (NNT): 10] between Groups I and II, 63.5% (NNT: 2) between Groups I and III and 52.8% (NNT: 2) between Groups II and III (B).^{21,22}

Twenty-six consecutive patients with newly diagnosed APL or in the first relapse, with ages ranging from 14 to 54 years, were evaluated regarding the improvement of the coagulopathy after starting treatment with oral ATRA (25 to 30 mg/m²/day) or an intravenous infusion of 0.1% arsenic trioxide (As₂O₃ - 10 mL/day). This analysis was made in the bone marrow by studying the release of procoagulant substances by blastic cells; a return to normality was detected 14 days after the start of treatment (*p*-value < 0.001). In the first week of treatment, the bleeding symptoms improved in both the ATRA and As₂O₃ Groups and the plasma fibrinogen concentration presented progressive and significant increases (*p*-value < 0.01 and *p*-value < 0.05, respectively) with return to normality. Moreover, the platelet alpha-granule membrane proteins (GMP-140), which were elevated prior to starting treatment, decreased significantly by the seventh day (*p*-value < 0.05 in both groups), with return to normality in the evaluation by the 14th day of treatment; the levels of soluble fibrin monomer complex (SFMC) and D-dimer, which were high at the beginning, showed significant reductions in both groups (*p*-value < 0.001) at the end of one week. However, the levels of SFMC were normal for the As₂O₃ Group at 14 days and only at 21 days for the ATRA Group. The plasma D-dimer concentrations remained high until CR, but without hemorrhagic manifestations throughout the period (B).²³ In the analysis of the plasma levels of natural anticoagulants and fibrinolytic proteins, protein C and protein S were low at the beginning of treatment but there was a significantly higher increase in protein C activity in the ATRA Group compared to the As₂O₃ Group (*p*-value < 0.001 and *p*-value < 0.01, respectively), accompanied by a slight increase in plasma protein C. The plasma thrombomodulin concentration, elevated at diagnosis, showed significant reductions in both groups (*p*-value < 0.001). The increase in tissue factor plasma inhibitor (TFPI) decreased during treatment, without any difference in the fibrinolytic system (plasminogen activity, α₂-plasminogen inhibitor, tissue plasminogen activator, plasminogen activator inhibitor) in both groups (B).²³

The frequency of thrombotic events was compared between groups of patients treated with a regimen based on a combination of ATRA with an anthracycline or chemotherapy alone. In a retrospective study, thirty-one patients with newly diagnosed APL were treated with ATRA (45 to 50 mg/m²/day) associated with idarubicin (12 mg/m²/day) for four days starting on the fifth day of treatment or before in cases of a leukocyte count > 10 × 10⁹/L. The comparison was made with a group of 25 patients treated with systemic chemotherapy alone before ATRA was on the market. There was 16% of thromboembolic phenomena in both treatment groups, with

6.5% of fatal bleeding in the group treated with ATRA plus chemotherapy and 8% in the group treated with systemic chemotherapy, without significant difference (B).^{22, 24,25}

The role of heparin in the management of coagulopathies associated with APL was evaluated in a retrospective study of 115 patients with a median age of 36.7 years (range: 3-73 years). Patients were treated with systemic chemotherapy alone, not associated with ATRA. According to the decision of the attending physician, heparin was used in varying doses and routes of administration: (subcutaneous 5000 U b.i.d. or intravenous 2400 to 24,000 U/day). Fresh frozen plasma, cryoprecipitate and platelet concentrates were used as needed (B).²⁶ CR was seen for 60% of the patients with 86% in the group with heparin and 49% in the group without heparin (*p*-value = 0.002). This difference was attributed to the reduction in the number of fatal hemorrhages, especially intracranial bleeding, in the group undergoing treatment with heparin (2.6% vs. 22.6%; *p*-value < 0.005) (B).²⁶

Recommendations

The recommended treatment for bleeding disorders in patients with APL is the immediate start of ATRA therapy with support using blood products to keep the platelet count above 30 × 10⁹/L and the fibrinogen concentration above 100 mg/dL (using concentrated platelet, fresh frozen plasma and cryoprecipitate transfusions). There is no evidence of benefits with the use of heparin.

Which anthracycline should be used in induction therapy for acute promyelocytic leukemia in terms of response rate, overall survival and toxicity?

P – Acute promyelocytic leukemia

I – Anthracycline (daunorubicin, doxorubicin, idarubicin or mitoxantrone)

O – Response rate, overall survival and toxicity

Eighty patients with morphologic diagnosis of APL were treated during the period from 1963 and 1971. Thirty-six patients were treated before 1967 with 6-mercaptopurine (6MP) and prednisone or 6MP, methotrexate and prednisone or methylglyoxal-bis (guanyldrazone - methyl-GAG). The remaining 44 were treated after 1967 with daunorubicin (60 mg/m²/day for four days) followed by a break of three days and another course of daunorubicin based on a cytological examination of the bone marrow. The CR rate was 13% for patients treated with other regimens and 55% for those who received daunorubicin. The median duration of CR was 15 days for the group that received other drugs and 25 months for the daunorubicin Group (A).²⁷

Sixty-eight patients with cytogenetic or molecular diagnosis of APL, aged 60 years or older, with normal liver and kidney function and without severe heart disease or infections at diagnosis were assessed in respect to response to induction therapy comprising one or two cycles of systemic chemotherapy associated with ATRA (45 mg/m²/day) until CR or for a maximum of 60 days [the German Acute Myeloid

Leukemia-Cooperative Group (AMLCG) Protocol] (B).²⁷ The first cycle consisted of 6-thioguanine (100 mg/m² b.i.d. orally on D3 to D8), cytarabine (100 mg/m²/day as a continuous infusion on D1 and D2 followed by 100 mg/m² b.i.d. on D3 to D8) and daunorubicin (60 mg/m²/day on D3 to D5). If the patient's doctor knew that the patient would tolerate a second induction cycle, this was conducted with cytarabine (1 g/m² b.i.d. from D1 to D3) and mitoxantrone (10 mg/m² on D1 to D3) (B).²⁷ The CR rate was 82% and the early death rate was 18%. The deaths occurred between 2 to 19 days after the beginning of induction therapy with the death rate being higher in patients with leukocyte counts > 10 × 10⁹/L (*p*-value = 0.009) and in patients over 70 years of age (*p*-value = 0.048). The main causes of death were bleeding, multiple organ failure and sepsis (B).²⁸

One hundred and one patients aged from 19 to 73 years with APL confirmed by cytogenetics or a molecular study underwent induction treatment with ATRA (45 mg/m²/day) until CR, idarubicin (12 mg/m² for under 61-year-old patients on D2, D4, D6 and D8 - 9 mg/m² for patients from 61 to 70 years old and 6 mg/m² for over 71-year-old patients), and prednisone (50 mg/day) if the WBC count was > 10 × 10⁹/L at diagnosis (APML3 Protocol); 8% died within the first 30 days of treatment with the main causes being bleeding, Differentiation syndrome, multiple organ failure and sepsis. Differentiation syndrome is formally known as ATRA syndrome. The CR rate was 90%. The 4-year DFS rate was estimated at 69.7% and 4-year OS was estimated at 83.7% (B).²⁹

The AIDA 0493 Protocol with ATRA (45 mg/m²/day) until CR or for a maximum of 90 days and idarubicin (12 mg/m² on D2, D4, D6 and D8) in the induction therapy and three cycles of consolidation with idarubicin and cytarabine (Cycle 1), etoposide and mitoxantrone (Cycle 2) and idarubicin, cytarabine and 6-thioguanine (Cycle 3) followed by randomization for maintenance was used in 828 under 75-year-old patients diagnosed with APL confirmed by cytogenetics or a molecular study. The CR rate was 94.03%; 5.45% died during induction and 0.25% of cases were considered resistant to initial treatment. The main causes of deaths during the induction therapy were bleeding (70.8%), infections (13.6%), thromboembolism (6.8%) and Differentiation syndrome (6.8%). The 12-year EFS and OS were estimated at 68.9% and 76.5%, respectively (B).³⁰

The PETHEMA Group evaluated 732 patients with APL confirmed by molecular biology and cytogenetics treated between 1996 and 2005. The treatment protocol included an induction phase, consolidation and maintenance. Induction consisted of ATRA (45 mg/m²/day) until CR or a maximum of 90 days and idarubicin (12 mg/m²/day on D2, D4, D6 and D8). Consolidation consisted of three cycles of ATRA and chemotherapy with idarubicin, mitoxantrone or cytarabine adapted to the patient's risk status. Maintenance was with 6MP, methotrexate and ATRA, and lasted for two years. The CR rate was 90.9% and the 5-year EFS and OS were estimated at 75% and 75%, respectively for the LPA96 Protocol and 83% and 81%, respectively for the LPA99 Protocol (B).¹⁸

The LPA93 Protocol consisted of ATRA (45 mg/m²/day) until CR or for a maximum of 90 days, cytarabine (200 mg/m²/day by continuous infusion) for seven days and daunorubicin (60

mg/m²/day) on D1, D2 and D3. After CR patients received two cycles of consolidation with daunorubicin and cytarabine, plus maintenance with 6MP and methotrexate for two years. The number of patients enrolled in the study was 576 with 92.5% achieving CR. In a median follow-up time of ten years, 61.7% of the patients remained in CR and the estimated 10-year survival was 77%. After maintenance therapy, 26 patients died in CR with two due to heart failure (B).³¹

The International Consortium on Acute Promyelocytic Leukemia evaluated 183 patients treated with a protocol similar to that adopted by the PETHEMA Group however idarubicin was replaced by daunorubicin. The dose of daunorubicin was equivalent to five times that of idarubicin used in the induction and consolidation phases of the protocol. The remission rate was 85% and the mortality rate during induction was 15%. The main causes of death were bleeding (48.1%), infections (25.9%) and Differentiation syndrome (18.5%). The estimated 2-year OS and EFS were 80% and 91%, respectively (B).²⁰

In the analysis of 19 patients with again APL who were treated with a modified AIDA Protocol with mitoxantrone (10 mg/m² on D2, D4, D6 and D8) replacing idarubicin, associated with ATRA (45 mg/m²/day) until CR, eight patients had early death and 11 showed hematological remission after induction with a DFS of 82% (C).³²

Recommendations

Although there is no evidence that compare the different anthracyclines used in the induction treatment of APL patients, daunorubicin and idarubicin have proven effective in respect to the CR and survival rates especially when associated with ATRA. There is no comparative data regarding late toxicity.

Is induction with arsenic trioxide superior to induction with all-trans retinoic acid plus anthracycline?

P – Acute promyelocytic leukemia

I – Arsenic trioxide or arsenicals

C – All-trans retinoic acid

Patients who received ATRA (25 mg/m²/day) until CR (Group 1) or As₂O₃ (0.16 mg/kg/day) until CR (Group 2) or a combination of ATRA and As₂O₃ at similar doses (Group 3) were evaluated (A).³³ CR rates for the three groups were statistically similar (Group 1 vs. Group 2: *p*-value = 0.548; Group 1 vs. Group 3: *p*-value = 0.972; Group 2 vs. Group 3: *p*-value = 0.520), with differences in time to obtain CR; the means were 40.5 days (range: 25-65 days), 31 days (range: 28-38 days) and 25.5 days (range: 18-35 days) for Groups 1, 2 and 3, respectively (Group 1 vs. Group 2: *p*-value = 0.0233; Group 1 vs. Group 3: *p*-value = 0.0003; Group 2 vs. Group 3: *p*-value = 0.002). This difference could not be attributed to the chemotherapy added during the induction scheme as no significant difference was found for patients who received chemotherapy comparing the three groups (Group 1 vs. Group 2: *p*-value = 0.582; Group 1 vs. Group 3: *p*-value = 0.431; Group 2 vs. Group 3: *p*-value = 0.825) (A).³³

In the evaluation of peripheral blood, the platelet count normalized first in Group 3 (median: 22 days) followed by Group 1 (median: 33 days; p -value = 0.03) and Group 2 (median: 33 days; p -value = 0.031), while the recovery times of hemoglobin and WBC were similar between groups (A).³³

However, in Group 3, the coagulation time and fibrinolysis were only slightly less (median: 10.4; range: 6-19 days) than in Groups 1 (median: 11.7; range: 7-22 days) and 2 (median: 12.6; range: 8-24 days), without significant difference (A).³³

On monitoring residual disease by RT-PCR with quantification of the PML-RAR α fusion after induction treatment, a significant difference was identified between the groups (Group 1 vs. Group 2: p -value = 0.013; Group 1 vs. Group 3: p -value = 0.0092; Group 2 vs. Group 3: p -value = 0.041). The combined therapy showed no significant increase in hyperleukocytosis when compared with monotherapy and the presence of hepatic dysfunction was not a reason to suspend treatment (A).³³ However, it should be pointed out that treatment in Group 2 was based on ATRA alone during induction, a conduct that differs from current protocols in which it is associated to an anthracycline.

In the evaluation of ATRA (25 to 45 mg/m²/day) associated with As₂O₃ (0.15-0.16 mg/kg/day) or As₂O₃ alone in patients with again or relapsed APL, no significant difference was found in the CR rate between combination therapy (89.8%) and single drug therapy (81.7%) with a mean reduction of 6.51 days (95% CI: -11.32 to -1.70; p -value = 0.008) to achieve CR with combination therapy (A).³⁴

Recommendations

There is no evidence of superiority of As₂O₃ compared to ATRA combined with an anthracycline in the induction treatment for APL.

What are the risk factors for the development of Differentiation syndrome?

P – Acute promyelocytic leukemia

I – Risk factors

O – Differentiation syndrome

Twenty nine patients aged 16 to 81 years with APL confirmed by a molecular study began induction treatment with ATRA (45 mg/m²/day) associated with idarubicin (12 mg/m²/day) on D3, D5, D7 and D9 and prednisone (0.5 mg/kg/day) on D1-D15 (or intravenous methylprednisolone in patients who were not able to receive oral medications); 48.3% of these patients presented Differentiation syndrome without any significant difference between patients with leukocytosis ($> 10 \times 10^9/L$) and those without leukocytosis (B).³⁵

The risk of developing Differentiating syndrome according to the body mass index (BMI) was assessed in 144 patients with newly diagnosed APL who received ATRA and idarubicin following the AIDA-0493 and Aida-2000 protocols of the GIMEMA Group. Four of the underweight (BMI < 18.5 kg/m²) and normal patients (BMI: 18-25 kg/m²) patients developed the syndrome compared to 21 overweight (BMI: 25-30 kg/m²) and

obese patients (BMI > 30 kg/m²) and so BMI was identified as an independent risk factor for the development of Differentiation syndrome (OR: 7.4; 95% CI: 1.0-34.5; p -value = 0.014) (B).³⁶

In the evaluation of 413 patients with newly diagnosed APL, 15% (95% CI: 12-19) developed Differentiation syndrome during the induction treatment. On comparing the leukocyte counts at diagnosis ($< 5 \times 10^9/L$, $5-10 \times 10^9/L$, $10-20 \times 10^9/L$ or $> 20 \times 10^9/L$) there was no significant difference between patients who developed the syndrome (B).³⁷

In a retrospective analysis of 102 patients diagnosed with APL between August 1993 and December 2007 who received ATRA (45 mg/m²/day) as induction treatment associated to conventional chemotherapy or not, 7.8% developed Differentiation syndrome after a median of nine days (range: 2-23 days) from the beginning of the treatment. Age, gender, morphological and molecular subtypes, WBC count at diagnosis $> 10 \times 10^9/L$ and chemotherapy were not statistically significant risk factors for the development of the syndrome (B).³⁸

Of 39 patients, with a mean age of 26 years diagnosed with PML and treated with ATRA and idarubicin according to the PETHEMA regimen, 30.5% developed Differentiation syndrome (seven severe, four moderate and three fatal cases) within an average of 12 days (range: 3-23 days) from the beginning of ATRA therapy. Of the patients with BMI ≥ 30 , 66.6% developed the syndrome compared with 18.5% of patients with BMI < 30 (p -value = 0.012). Other predictors of Differentiation syndrome in the univariate analysis were: age ≥ 40 years (p -value = 0.033), baseline leukocyte count $\geq 20 \times 10^9/L$ (p -value = 0.003) and creatinine > 1.4 mg/dL (p -value = 0.009). However, in the multivariate analysis, only BMI ≥ 30 (p -value = 0.044) and baseline leukocyte count $\geq 20 \times 10^9/L$ (p -value = 0.025) remained as independent predictors for the development of Differentiation syndrome (B).³⁹

In the evaluation of 739 patients with APL, 24.8% developed Differentiation syndrome, with 12.6% of severe and 12.2% of moderate cases. In multivariate analysis a baseline leukocyte count $> 10 \times 10^9/L$ (OR: 1.85; 95% CI: 1.3-2.7; p -value = 0.012) and creatinine above normal (OR: 5.8; 95% CI: 1.9-16.9; p -value = 0.004) were associated with increased risk of developing Differentiation syndrome; this was repeated in the univariate analysis for the baseline leukocyte count (p -value = 0.03) and above normal creatinine levels (p -value = 0.002) (B).⁴⁰

For patients between seven and 34 years of age treated with ATRA associated with systemic chemotherapy, there was a higher incidence of Differentiation syndrome in those with WBC counts at diagnosis $> 10 \times 10^9/L$, however, this was not statistically significant (p -value = 0.08) (C).⁴¹

Recommendations

Leukocytosis is a predictor for the development of Differentiation syndrome when the WBC count is $> 20 \times 10^9/L$. A BMI above 30 and creatinine above the normal value are predictors for the development of Differentiation syndrome. There is no evidence for age, gender, morphological and molecular subtypes and concomitant systemic chemotherapy as risk factors for the development of the syndrome.

Is the prophylactic use of corticosteroids in patients with acute promyelocytic leukemia able to prevent Differentiation syndrome?

P – Acute promyelocytic leukemia/Differentiation syndrome

I – Corticosteroid, prophylaxis

C – All-trans retinoic acid

O – Prevent Differentiation syndrome

Differentiation syndrome is the main complication associated with therapy using agents that promote differentiation, such as ATRA and As_2O_3 , during induction therapy of APL. ATRA is well tolerated, however the incidence of Differentiation syndrome has been described in up to 27% of cases of APL (B).^{37,38,42} In most reported cases, symptoms relating to Differentiation syndrome began in a median of nine days (range: 0-23 days) from the start of treatment (B).³⁸

In patients with APL treated with ATRA (45 mg/m²/day) initiated within the first 24 to 48 hours after diagnosis, 48.3% evolved Differentiation syndrome according to the criteria of Frankel (three of the following signs in the absence of other causes: fever, weight gain, pulmonary infiltrate, pericardial or pleural effusion, hypotension, liver failure and/or acute renal failure) with 37.9% of early deaths (B).⁴³

On suspicion of symptoms related to Differentiation syndrome, the early addition of high doses of dexamethasone reduces associated mortality from 30 to 5% or less (B).⁴⁴ It is recommended to start dexamethasone using an intravenous dose of 10 mg b.i.d. until total regression of the symptoms. Most patients present a rapid improvement with the administration of the corticosteroid. The preemptive use of corticosteroid early after the start of the clinical manifestations of Differentiation syndrome is the standard recommended treatment however its prophylactic use is still quite controversial. There is only one work of the Australian Group in which prednisolone (75 mg/day) was administered prophylactically in 12 of 19 (63%) patients being treated with ATRA; a reduction in pulmonary toxicity was demonstrated in patients with leukocyte counts $> 10 \times 10^9/L$ (B).⁴⁵ Based on this result, the Spanish group started three clinical trials to evaluate the impact of prophylaxis with corticosteroids in patients with APL and WBC counts $> 5 \times 10^9/L$ at diagnosis.⁴⁶ In the LPA96 study, all patients with leukocyte counts $> 5 \times 10^9/L$ before or during the institution of therapy with ATRA received prophylaxis with an intravenous dose of dexamethasone (10 mg b.i.d.) for seven days and the incidence of Differentiation syndrome was 30%. The LPA99 study advocated the use of prophylaxis with oral prednisone at a dose of 0.5 mg/kg/day from D1 to D15 of the protocol for all patients and observed an incidence of Differentiation syndrome of 23%. In the LPA2005 study, prophylaxis was administered only to patients with leukocyte counts $> 5 \times 10^9/L$. These patients received dexamethasone at a dose of 2.5 mg/m² b.i.d. during 15 days, resulting in 28% of Differentiation syndrome. A retrospective comparison of these three clinical trials showed a slight reduction in the incidence of Differentiation syndrome, however it showed no reduction in mortality related to this syndrome (B).^{41,47}

Recommendations

So far, there is no evidence supporting the prophylactic use of corticosteroids in patients with APL to prevent Differentiation syndrome.

What is the best therapeutic conduct for Differentiation syndrome?

P – Differentiation syndrome

I – Corticosteroid, diuretics, interruption of ATRA

O – Improvement in symptoms

In a retrospective study of a series of 102 patients with APL treated initially with ATRA (45 mg/m²/day), eight patients (7.8%) presented Differentiation syndrome based on the clinical manifestations following the criteria of Frankel. Symptoms suggestive of the syndrome began in a median of nine days after starting treatment (range: 2-23 days) with the most common being fever (87.5%) and respiratory dysfunction (87.5%) (B).³⁸

There was no statistically significant difference for gender and age, morphological variant and the isoforms of PML-RAR α fusion gene transcript in patients who developed Differentiation syndrome compared to those who did not. None of the patients with APL and a clinical picture suggestive of Differentiation syndrome had a leukocyte count $> 10 \times 10^9/L$ at diagnosis and there was no relationship between patients treated with ATRA alone or combined with systemic chemotherapy (p -value = 1.00) (B).³⁸ In this series, Differentiation syndrome improved after the interruption of ATRA associated with intravenous dexamethasone (20 mg b.i.d.) until total regression of symptoms.

In the experience of the PETHEMA group, one quarter of the patients with APL under treatment with ATRA developed Differentiation syndrome within 7 to 12 days after starting the induction phase of treatment. All patients presented with respiratory disorders, unexplained fever, weight gain, edema, pleural effusion and pulmonary infiltrate. The most serious Differentiation syndrome occurred early, on average in six days accompanied by acute renal failure. The diagnosis of Differentiation syndrome was established by clinical manifestations, according to the criteria of Frankel, associated with radiological findings of pulmonary infiltrate. The recommendation of the Spanish group for the management of Differentiation syndrome is the immediately introduce intravenous dexamethasone (20 mg/day) and diuretics until improvement of the symptoms; ATRA should be discontinued in cases of severe respiratory dysfunction syndrome, hypotension or renal failure (B).⁴²

In the evaluation of 413 patients with APL, confirmed by the presence of the t (15; 17), 64 patients (15%; 95% CI: 12-19) developed Differentiation syndrome during the induction treatment with the onset of symptoms from one to 35 days after beginning the treatment (median: 7 days) (B).³⁷

The most frequent manifestations were respiratory dysfunction (89%), fever (81%), pulmonary infiltrate (81%), weight gain (50%) pleural effusion (47%), kidney disease (39%)

pericardial effusion (19%) and hypotension (12%). On suspicion of the diagnosis of Differentiation syndrome, 84.37% of the patients received intravenous dexamethasone (10 mg b.i.d.) for three to 23 days (median: six days) with suspension of ATRA in the most severe cases (46.87%). The Differentiation syndrome-related mortality rate was 8% in this study. Fifty-five patients (86%) with the syndrome achieved CR compared with 94% of the group without the syndrome (B).³⁷

Differentiation syndrome occurred in 183 (24.8%) of the 739 cases of the LPA96 and LPA99 studies. In 93 patients (12.6%) symptoms were severe in particular with respiratory dysfunction (dyspnea) in 95% of cases, accompanied by pulmonary infiltrate, fever, kidney injury and pleural effusion. ATRA was suspended in 60% of the cases of the syndrome (p -value < 0.001); dexamethasone (10 mg b.i.d.) was prescribed for 90% of the patients (p -value < 0.001) and diuretics for 87% (p -value < 0.001). Dialysis was performed in 12% of the cases (p -value = 0.003) and mechanical ventilation in 26% (p -value = 0.002). There were 26% of deaths during the induction treatment with 11% due to Differentiation syndrome (B).⁴⁰

In the evaluation of 29 patients with APL with a mean age of 48 years treated with ATRA (45 mg/m²/day) initiated in 24 to 48 hours after diagnosis, 48.3% developed Differentiation syndrome according to the criteria of Frankel (B).⁴³ In this series, 10 of 14 (71.4%) patients suffered acute renal failure and of these 60% required dialysis. Kidney failure contributed to the mortality. The author suggests that therapy with corticosteroid (dexamethasone – 20 mg/day) and the suspension of ATRA are effective measures against Differentiation syndrome.

The consensus of experts from the European LeukemiaNet recommends that intravenous dexamethasone (20 mg b.i.d.) should be started immediately on suspicion of any one of the signs or symptoms of Differentiation syndrome (dyspnea, unexplained fever, weight gain, peripheral edema, hypotension, renal failure, congestive heart failure, and particularly a chest x-ray showing lung infiltrate or pericardial effusion) (D).⁸

Recommendations

On suspicion of Differentiation syndrome, immediately start intravenous dexamethasone (20 mg b.i.d.) and use for at least three days or until total regression of the symptoms. Diuretics should be used to maintain a neutral or negative water balance. Suspension of ATRA is indicated only in the most serious cases with need of treatment in the intensive care unit for respiratory distress (dyspnea and hypoxemia), acute renal failure or hypotension. ATRA should be interrupted for 48-72 hours and reintroduced at a 50% dose with stepped increases until the full dose.

What is the best therapeutic conduct for pseudotumor cerebri syndrome?

P – Pseudotumor cerebri syndrome in patients with acute promyelocytic leukemia
I – Corticoid

Pseudotumor cerebri is characterized by increased intracranial pressure with the presence of papilledema, normal or diminished ventricles and with normal cerebrospinal fluid (CSF). Diagnosis is by the modified Dandy criteria which includes signs and symptoms of raised intracranial pressure, lack of localizing findings in the neurological examination, the absence of deformities or obstructions of the ventricular system or any other change in the neuroimaging study, except for changes in visual acuity and eye movement and increased CSF pressure with no other causes of increased intracranial pressure (C).⁴⁸

Among the drugs that are associated with the appearance of pseudotumor is ATRA; this adverse effect more commonly appears in children (C).^{48,49}

In the evaluation of patients with de novo or relapsed APL two days to six months after starting treatment with ATRA, the following signs and symptoms suggest a diagnosis of pseudotumor cerebri: appearance of symptoms suggestive of increased intracranial pressure such as headaches, blurred vision, diplopia, nausea and vomiting, with bilateral papilledema, normal computed tomography and magnetic resonance imaging results and a lumbar puncture with CSF with absence of cells and normal glucose and protein concentrations, but with increased pressure (C).⁵⁰⁻⁶²

In all cases there was an improvement of symptoms in two to 28 days after the suspension of ATRA (C).⁵⁰⁻⁶²

There is a description of the use of mannitol and acetazolamide together with the suspension of ATRA to decrease the CSF pressure improving the headaches, diplopia, nausea and vomiting in two days and improvement of papilledema in seven days (C).⁶² Moreover there is a description of removal of CSF without suspension of ATRA which improved the symptoms (C).⁵⁴

Recommendations

The temporary suspension of ATRA is recommended when there is diagnostic suspicion of pseudotumor cerebri in patients with APL. ATRA can be reintroduced after improvement of the symptoms.

Is prophylaxis of the central nervous system indicated for a specific group of patients with acute promyelocytic leukemia?

P – Acute promyelocytic leukemia
I – Methotrexate or cytarabine
O – Prophylaxis of the central nervous system

Although the relapse rate in the CNS is low (0.6-2.0%), it is the most common extramedullary site for relapse of APL and at least 10% of relapses involve in the CNS (D).⁶³

In the evaluation of adult patients with relapse of APL with leptomeningeal involvement (without prior CNS involvement), the initial treatment is a dose of triple intrathecal therapy (methotrexate, cytarabine and hydrocortisone) on the day of the diagnostic lumbar puncture before starting therapy

with liposomal cytarabine associated with dexamethasone to prevent arachnoiditis (C).⁶⁴

In a follow-up of 70 months, 23% of adult APL patients in CR relapsed, with 6% involving extramedullary sites. Of the cases of relapse in extramedullary sites, 90% involved the CNS and 10% the skin. The median WBC count at diagnosis of these patients was $26.95 \times 10^9/L$ (range: $7.7\text{--}162 \times 10^9/L$), with 80% having counts above $10 \times 10^9/L$, 30% with APL variants, and in the evaluation of the breakpoint of the PML-RAR α fusion transcript, 30% had the bcr-1 isoform and 60% the bcr-3 isoform. Bone marrow involvement is documented in 89% of relapses in the SNC (B).⁶⁵

A univariate analysis in this same study identified the following risk factors related to extramedullary relapse: age less than 45 years ($p\text{-value} = 0.05$), the presence of the PML-RAR α bcr-3 isoform ($p\text{-value} = 0.0003$) and high WBC count at diagnosis ($p\text{-value} < 0.0001$). Differentiation syndrome was not associated with increased risk of extramedullary relapse ($p\text{-value} = 0.69$) (B).⁶⁵ In multivariate analysis, only the high WBC count at diagnosis ($p\text{-value} = 0.0014$) was associated with relapse at an extramedullary site. However, whether to routinely perform prophylaxis or not of the SNC against relapse using intrathecal chemotherapy or high-dose ARA-C in patients with leukocyte counts above $10 \times 10^9/L$ at diagnosis is not yet completely defined (B).⁸ In protocols where prophylaxis is made with intrathecal chemotherapy, this must be carried out after obtaining a CR as a lumbar puncture at diagnosis and during induction can be extremely risky (D).⁸

Another study that evaluated 739 patients treated with idarubicin and ATRA also showed that relapse-free survival in patients with or without Differentiation syndrome is similar. None of the ten patients who had relapsed in the SNC presented with Differentiation syndrome (B).⁴⁰

In the APL2000 study of the European Group, under 60-year-old patients with leukocyte counts $> 10 \times 10^9/L$ received a higher dose of ARA-C and prophylaxis of the SNC [five applications of methotrexate (15 mg), ARA-C (50 mg) and corticosteroid IT] in the second consolidation cycle and over 60-year-old patients received prophylaxis of the SNC alone. The rate of relapse in the under 60-year-old group was 2.9% and there was no relapse in the SNC (B).⁶⁶

Recommendations

There is no evidence to recommend prophylaxis of the CNS in APL patients.

Are there benefits with the use of cytarabine in acute promyelocytic leukemia?

P – Acute promyelocytic leukemia

I – Cytarabine

O – Complete remission, disease-free survival, overall survival

ATRA in association with an anthracycline is the standard treatment for the induction phase of the treatment of APL (2A).⁸ In the study carried out by Adès et al., under 60-year-

old patients diagnosed with APL and with WBC counts $< 10 \times 10^9/L$ were randomized to two groups.⁶⁷ Standard induction treatment included ATRA (45 mg/m²/day) until CR in combination with daunorubicin (60 mg/m²/day) for three days. For one group, cytarabine (200 mg/m²/day) for seven days was associated starting on the third day after the beginning of ATRA administration. In a 35-month follow-up, the CR rate was 96.5%. In the group that received cytarabine the CR was 99% and for the group without cytarabine it was 94%, without statistical significance ($p\text{-value} = 0.12$) (A).⁶⁷ The molecular remission rate was 90% in the group that received cytarabine and 82% for patients who did not ($p\text{-value} = 0.18$) (A).⁶⁷ These results were corroborated by the PETHEMA study of the Spanish group that, even though it did not investigate the effect of the association of cytarabine to standard induction treatment (ATRA and anthracycline), reported CR rates of between 90% and 95% in patients with APL treated exclusively with ATRA and anthracycline (C).^{18,68} The remission rate reported by the international consortium for Acute promyelocytic leukemia in 180 Brazilian, Chilean, Mexican or Uruguayan patients treated with ATRA (45 mg/m²/day until CR) and daunorubicin (60 mg/m²/day on D2, D4, D6 and D8) was 85% (C).³¹

With regard to consolidation therapy for APL, most studies after the 1990s followed the concept of treatment adapted to the risk of relapse (A).^{18,30,69,70} Patients are classified at diagnosis as low, intermediate or high risk for relapse according to the leukocyte and platelet counts. The proposal that is most widely used is that reported by the PETHEMA and GIMEMA studies: low-risk patients have leukocyte counts $< 10 \times 10^9/L$ and platelet counts $\geq 40 \times 10^9/L$, intermediate-risk patients have leukocyte counts $< 10 \times 10^9/L$ and platelet counts $< 40 \times 10^9/L$, and high-risk patients have leukocyte counts $> 10 \times 10^9/L$.^{18,30}

In under 60-year-old patients with APL in CR after induction treatment with ATRA and idarubicin, the association of cytarabine or not was compared in the consolidation therapy of high-risk patients; the group that did not receive cytarabine was followed up for 82 months and the group that received the medication was followed up for 28 months (A).⁷⁰ Three cycles of consolidation therapy were used, with the second cycle with mitoxantrone (10 mg/m²/day) for five days being similar in the two groups. In the first cycle, Group 1 received idarubicin (7 mg/m²/day) for four days and Group 2 received idarubicin (5 mg/m²/day) associated with cytarabine (1 g/m²/day) for four days. In the third cycle, Group 1 received idarubicin (12 mg/m²/day) for two days and Group 2 received idarubicin (12 mg/m²/day) for one day associated with cytarabine (150 mg/m² t.i.d.) for four days. Fifteen days of ATRA (45 mg/m²/day) was included in all three cycles (A).⁶⁷ Although thrombocytopenia and neutropenia were more common in Group 2 that received cytarabine ($p\text{-value} < 0.001$), there was no significant difference in CR (81% vs. 83%, respectively) or OS (71% vs. 79%, respectively; $p\text{-value} = 0.34$). However, the cumulative incidence of relapse at three years was significantly lower in the group that received cytarabine (26% vs. 11%, respectively; $p\text{-value} = 0.03$) (A).⁷⁰

The German group studied the role of cytarabine in treating under 60-year-old patients with APL. Patients were treated with

idarubicin and ATRA in the induction phase and evaluated in the consolidation phase on a cycle of idarubicin (12 mg/m²/day) on D1, D3 and D5, cytarabine (100 mg/m²/day) as seven days of continuous infusion and etoposide (100 mg/m²/day) for three days and two cycles of cytarabine (3 g/m² b.i.d.) for three days associated with mitoxantrone (10 mg/m²/day) for two days (B).¹⁸ The CR rate was 88% and the mortality rate during treatment was 12%. The DFS and OS, after a follow-up period of 46 months, were 83% and 82%, respectively (B).⁷¹

Recommendations

The association of cytarabine to an anthracycline and ATRA in the consolidation therapy of patients diagnosed with APL is recommended for under 65-year-old high-risk patients based on the significant reduction in the relapse rate and the significant increases in EFS and OS. There is no evidence to support this association for intermediate- or low-risk patients.

What is the role of transplantation in relapse of acute promyelocytic leukemia and when should allogeneic and autologous transplants be indicated?

P – Acute promyelocytic leukemia

I – Allogeneic transplant

C – Autologous transplant

O – Complete remission, disease-free survival, overall survival

In a retrospective study, 73 of 122 (60%) patients with relapsed APL who achieved a second complete hematological remission underwent bone marrow transplantation (BMT). Fifty patients (median age: 45 years) were submitted to autologous BMT and 23 (median age: 32.5 years) to allogeneic BMT. For those who underwent autologous BMT, the DFS, EFS and OS at seven years were 79.4%, 59.4% and 60.6%, respectively, with 6% of transplant-related deaths, 2% of deaths due to lung cancer 49 months after the BMT, 6% of relapse three to 18 months after the BMT and 4% of myelodysplastic syndromes (SMD) (B).⁷² For patients who underwent allogeneic BMT, the DFS, EFS and OS at seven years were 92.3%, 52.2% and 51.8%, respectively, with 39% of transplant-related deaths, 4.3% of deaths from non-Hodgkin's lymphoma and 4.3% of relapse after 17 months of the BMT (B).⁷² Forty-nine patients (median age: 45 years) were treated with systemic chemotherapy after the second remission with 10.2% of treatment-related deaths and 53% of relapse after two to 22 months. In the case of systemic chemotherapy the DFS, EFS and OS were 42.7%, 37.4% and 46%, respectively (B).⁷² The OS was significantly higher for patients who underwent autologous BMT (p -value = 0.04) while there was no significant difference for DFS and EFS between autologous or allogeneic BMT (p -value = 0.19 and p -value = 0.11, respectively). The DFS, EFS and OS were also significantly higher in patients who underwent autologous BMT compared to patients who received systemic chemotherapy (p -value = 0.002, p -value = 0.0005 and p -value = 0.001, respectively). The EFS and OS were similar in the groups of patients who received

allogeneic BMT and systemic chemotherapy (p -value = 0.17 and p -value = 0.41, respectively), but the DFS was better in those who received allogeneic BMT (p -value = 0.0018) (B).⁷²

These data suggest that autologous BMT is very effective in the treatment of APL for patients in second complete molecular remission. Allogeneic BMT, though with a lower relapse rate, is associated with high transplant-related mortality (B).⁷²

The European Group for Bone Marrow Transplant evaluated 625 patients with APL after 1993 who received autologous or allogeneic BMT after the first or second CR. The DFS at five years was 69% for the 149 patients in first remission who underwent autologous BMT and 68% for the 144 who underwent allogeneic BMT. The DFS after the second remission was 51% for the 195 patients who were submitted to autologous BMT and 59% for the 137 patients who underwent allogeneic BMT. The data lead to the conclusion that transplants still seem to have a role to play in APL, especially for patients in second remission (B).⁷³

A retrospective analysis was performed of 32 patients aged between one and 16 years diagnosed with relapsed APL or disease refractory to the first treatment and who underwent autologous (n = 11) or allogeneic BMT (n = 21). In the case of autologous BMT, treatment-related mortality was 0% (95% CI: 0-30) and the relapse rate was 27% (95% CI: 9-57) and for allogeneic BMT, treatment-related mortality was 19% (95% CI: 7-41) and the relapse rate was 10% (95% CI: 2-30). The mean times to relapse were 15.3 months (range: 4.4-42.7 months) and 13.5 months (range: 7.3-19.6 months) for autologous and allogeneic BMT, respectively. The EFS at five years was 73% for autologous BMT and 71% for allogeneic BMT (p -value = 0.81) and the OS was 82% and 76%, respectively (p -value = 1.0) (B).⁷⁴

The European Group of Hematopoietic Stem Cell Transplantation recommends autologous BMT or a transplant with a HLA-identical related donor for cases of APL in second CR and BMT with a HLA-identical related donor in the case of molecular persistent APL (D).⁷⁵

Recommendations

In APL, autologous hematopoietic stem cell transplant is accepted for patients in second complete molecular remission.

How to make the diagnosis of molecular relapse? Which method, quantitative or qualitative polymerase chain reaction, is most recommended?

P – Acute promyelocytic leukemia

I – Quantitative polymerase chain reaction,

C – Qualitative polymerase chain reaction

O – Molecular relapse

For patients with APL initially treated with As₂O₃ (27.2% low-risk and 72.8% high-risk), RT-PCR and quantitative real time polymerase chain reaction (RQ-PCR) was performed at diagnosis, at the end of induction treatment, at the beginning of the consolidation phase and every three months during the two years of maintenance treatment with subsequent evaluation for the presence of PML-RAR α transcripts to assess

minimal residual disease (MRD) and its predictive effect on relapse (B).⁷⁶

For both groups, the results were positive for PML-RAR α using RT-PCR and RQ-PCR in 100% of cases at diagnosis, 63.82% and 85.18%, respectively at the end of the induction treatment (at this time there was 100% of complete hematological remission), 19.49% and 34.78%, respectively before the beginning of the consolidation treatment and 0% and 27.11%, respectively during maintenance treatment (B).⁷⁶

In multivariate analysis, there was a 4.8-fold higher risk of relapse in patients with positive RT-PCR results at the end of the induction treatment compared to patients negative for the PML-RAR α (95% CI: 1.13-21.19; *p*-value = 0.034), without significant impact on the risk of relapse at the beginning of the consolidation. The sensitivity, specificity and VR that a positive RT-PCR predicted relapse after induction were 86.7%, 42.3% and 1.50, respectively; and the sensitivity, specificity and VR that a positive RQ-PCR predicted relapse were 89.3%, 19.2% and 1.10, respectively (B).⁷⁶

On comparing FISH with RT-PCR for the diagnosis and monitoring of APL, failures to identify disease were seen in 8% and 4%, respectively at diagnosis, in 38% and 13%, respectively at the end of induction and in 66% and 0%, respectively at the end of the consolidation treatment. All cases that were negative by RT-PCR and FISH at the end of the induction and consolidation remained in molecular remission over 18 months of follow-up. The predictive values for relapse at the end of induction in patients with positive and negative RT-PCR results were 60 \pm 11% and 3%, respectively; for the positive and negative FISH testing results, these values were 57 \pm 13% and 6%, respectively (B).⁷⁷

In assessing the MRD in APL with RQ-PCR, values $\geq 1 \times 10^{-3}$ after the end of consolidation treatment compared with values $< 1 \times 10^{-3}$ proved to be the most important predictor of relapse (85.7 \pm 13.2% vs. 7.3 \pm 4.1%; *p*-value < 0.001) and DFS (14.3 \pm 13.2% vs. 91.2 \pm 4.3%; *p*-value < 0.001) (B).⁷⁸

The molecular response of patients with APL was evaluated by RT-PCR and RQ-PCR after induction and consolidation treatment. For RT-PCR, positive and negative results were defined as the presence or absence of PML-RAR α transcripts and for RQ-PCR, a minor response was defined when the level was between log 3.0 and 4.9 and a major response when the level was \geq log 5.0 (B).⁷⁹

After completion of the induction treatment, negative responses by RT-PCR and minor and major molecular responses by RQ-PCR were 35.5%, 22.6% and 12.9%, respectively. After consolidation treatment these responses were 96.8%, 96.8% and 90.3%, respectively. The time of treatment to attain negative results by RT-PCR was on average two months compared to three months to acquire a minor molecular response by RQ-PCR (*p*-value = 0.56) and four months to acquire a major molecular response (*p*-value = 0.0036) (B).⁷⁹

No association between a positive RQ-PCR result and relapse of the disease were found for patients aged between eight and 84 years with molecular diagnosis of APL after induction treatment. However, after the third cycle of consolidation, 66% of cases with positive RQ-PCR results relapsed compared to 13% of patients with negative RQ-PCR results. Around 23% of the cases that presented with hematologic relapse were not

predicted by molecular techniques (RT-PCR or RQ-PCR). In all these cases, consistent analysis had been performed four to five months before relapse (mean: 260; range: 153-386 days) (B).⁸⁰

Recommendations

Molecular diagnosis of relapse in APL can be performed by FISH, RT-PCR and RQ-PCR; RQ-PCR is the most important to predict hematologic relapse and DFS.

What are the best therapeutic options for hematologic and extramedullary relapse?

P – Relapse or recurrence and acute promyelocytic leukemia

I – All-trans retinoic acid or arsenic trioxide or systemic chemotherapy

O – Remission

Relapse was seen in 23% of cases of adult patients with APL in CR; 6% were extramedullary in a follow-up of 70 months. Of the relapse cases in extramedullary sites, 90% involved the CNS and 10% involved the skin. The median WBC count of these patients at diagnosis was $26.95 \times 10^9/L$ (range: 7.7-162 $\times 10^9/L$) with 80% having counts $> 10 \times 10^9/L$, 30% with APL variants, and in the evaluation of the breakpoint of the PML-RAR α fusion transcript, 30% have the bcr-1 isoform and 60% the bcr-3 isoform. Bone marrow involvement is documented in 89% of the relapses of the SNC (B).⁶⁵

A univariate analysis in this same study identified the following risk factors related to extramedullary relapse: age less than 45 years (*p*-value = 0.05), the presence of the PML-RAR α bcr-3 isoform (*p*-value = 0.0003) and high WBC count at diagnosis (*p*-value < 0.0001). Differentiation syndrome was not associated with increased risk of extramedullary relapse (*p*-value = 0.69) (B).⁶⁵ In the multivariate analysis, only the high WBC count at diagnosis (*p*-value = 0.0014) was associated with relapse at an extramedullary site and so, for prophylaxis against CNS involvement with intrathecal chemotherapy is indicated in cases of leukocyte counts $> 10 \times 10^9/L$ at diagnosis (B).⁶⁵

The treatment for relapse in SNC included triple intrathecal therapy in all cases associated with ATRA in 55.5% of the patients. Systemic chemotherapy was added as rescue therapy in 88.9% of the cases: an anthracycline (mitoxantrone or idarubicin) with high- or intermediate-dose cytarabine. Eleven percent of the patients with CNS involvement who entered in second CR performed irradiation as consolidation of the retreatment. The 10% of cases who had skin involvement performed irradiation without systemic chemotherapy, followed by maintenance treatment using low-dose cytarabine and intermediate-dose ATRA (B).⁶⁵

Of all the cases of relapse, 30% died due to bleeding from the CNS or sepsis during rescue treatment and 70% attained a second CR. Of these, 30% underwent allogeneic BMT and 30% autologous BMT. The median survival of patients with extramedullary relapse was 6.7 months compared to 26.3 months when relapse involved the bone marrow in isolation (*p*-value = 0.04) (B).⁶⁵

Patients in first relapse of APL previously treated with ATRA and systemic chemotherapy with anthracycline were compared with regard to treatment with a 3-hour intravenous infusion of As_2O_3 (0.15 mg/kg/day) in isolation or associated with oral ATRA (45 mg/m²/day) started on the first day of the administration of the As_2O_3 until the CR. In patients who presented leukocyte counts $> 30 \times 10^9/\text{L}$, daunorubicin (60 mg/m²/day) or amsacrine (90 mg/m²/day) for three days was associated to the regimen. With no significant difference between the groups, 80% of the patients obtained a second CR. However, all patients (10%) who did not obtained remission, were treated with As_2O_3 and ATRA and the remaining 10% died during treatment (A).⁸¹

Recommendations

Intrathecal chemotherapy associated with ATRA or As_2O_3 and systemic chemotherapy with cytarabine and anthracycline are recommended when relapse of APL involves the CNS. If the relapse is only hematological, treat with ATRA or As_2O_3 associated with cytarabine and anthracycline.

How should the patient with acute promyelocytic leukemia during pregnancy be treated?

P – Pregnant women with acute promyelocytic leukemia

I – All-trans retinoic acid or cytarabine or anthracycline (daunorubicin or idarubicin)

O – Complete remission, disease-free survival, overall survival

APL has been described in about 10% of cases of leukemia during pregnancy, similar to the percentage of leukemias in non-pregnant women (D).⁸²

Cases of women diagnosed with APL during gestation have been reported and analyzed separately during each trimester, taking into account the gestational age of diagnosis, the presence of disseminated intravascular coagulation (DIC), the treatment regimen employed, the gestational age at vaginal birth or C-section, if there were complications to the fetus and newborn, incidence of miscarriages and malformations (C).⁸³

Of the women evaluated, 28.6% were in the first trimester of pregnancy, 50% in the second trimester and 21.4% in the third trimester. All began treatment soon after diagnosis with varying schemes: ATRA alone, ATRA associated to an anthracycline and/or cytarabine, and/or other systemic chemotherapy or chemotherapy without ATRA (C).⁸³⁻⁹²

Of pregnant women diagnosed in the first trimester, 41.7% suffered miscarriages or abortions (for malformations), 50% delivered by C-section or vaginal and 8.3% had no details, with 50% of these women having been treated with ATRA alone or in association with systemic chemotherapy. Of the women diagnosed in the second trimester of pregnancy, 76.2% delivered by C-section or vaginal birth, 4.8% had miscarriages and there were no reports on 19%, with 57.1% having been treated with ATRA in isolation or in association with systemic chemotherapy. Of the pregnant women of third trimester, all

100% had C-sections or normal births and 77.8% were treated with ATRA alone (D).⁸³⁻⁹² All women showed significant improvement of the management of bleeding disorders at varying degrees after the first week of treatment, especially those receiving ATRA in isolation or associated with other medications (C).⁸³⁻⁹²

Of all patients, regardless of the gestational age at diagnosis, 83% achieved CR after induction treatment (C).⁸³

Due to the teratogenic potential of ATRA and As_2O_3 , both should be avoided during the first trimester of pregnancy and As_2O_3 throughout the pregnancy. Administration of chemotherapy during the first trimester, although it may be safe, is also associated with fetal malformations, increased risk of miscarriage and low birth weight (C).⁹³ The decision at this stage of pregnancy is to interrupt the pregnancy while there is hemodynamic stability to start treatment with ATRA in combination with chemotherapy or to continue with the pregnancy and prescribe anthracycline, starting with ATRA earlier in the second quarter. In this case the recommended anthracycline is for daunorubicin as it is effective in APL and there is more experience of its use during pregnancy (D).^{8,94}

The use of ATRA is relatively safe for mother and fetus in the second and third trimesters⁹³ (C) and there is no evidence of fetal malformations, although reversible fetal arrhythmias and other heart complications have been described at birth and so fetal monitoring is recommended during treatment (D).⁹⁵ At this stage of pregnancy, you can opt for sequential use of ATRA followed by chemotherapy, which would be launched after the end of the pregnancy, however taking into account the increased risk of the development of differentiation syndrome (approximately 25% of cases) (C).⁹⁶ Another option is the simultaneous use of ATRA and chemotherapy, which brings higher chances of cure and is a good option for high-risk patients with Leukocytosis (D).⁹⁴

Recommendations

Avoid the use of As_2O_3 throughout pregnancy and the use of ATRA in the first trimester of pregnancy. Daunorubicin is recommended in the first trimester. The use of ATRA during the second and third trimester of pregnancy seems effective to reverse coagulopathies and obtain CR without evidence of teratogenicity; it can be associated to chemotherapy or not.

How should an elderly patient with acute promyelocytic leukemia be treated?

P – Acute promyelocytic leukemia, elderly

I – All-trans retinoic acid or cytarabine or anthracycline (idarubicin or daunorubicin)

O – Complete remission, disease-free survival, overall survival

Patients aged 60 to 81 years (median: 65.5 years) with diagnosis of APL were treated with ATRA (45 mg/m²/day) associated or not with idarubicin (12 mg/m²/day) for four days; CR was obtained in 87.5% of cases with a mortality rate

of 12.5%. One to three cycles of consolidation treatment and maintenance with 6MP plus methotrexate was then carried out. In a median follow-up time of 19 months (range: 7-64 months), the OS and EFS at 24 months were 81.2% and 58.3%, respectively (B).⁹⁷

Patients with again APL aged 60 years or more with t (15; 17) or PML-RAR α rearrangement, normal liver and kidney function and without cardiac contraindications for the use of anthracyclines, were assessed regarding response to chemotherapeutic treatments (B).⁹⁸ Induction therapy consisted of ATRA (45 mg/m²/day) until the CR associated with idarubicin (12 mg/m²/day) on D2, D4, D6 and D8 in the first 36 months of follow-up (LPA96) or on days D2, D4 and D6 in the following 48 months (LPA99). Patients in CR were given three cycles of consolidation chemotherapy. The first cycle consisted of idarubicin (5 mg/m²/day) for four days, the second was with mitoxantrone (10 mg/m²/day) for five days and the third was with idarubicin (12 mg/m²/day) for one day. Patients who became PML-RAR α negative began maintenance therapy with mercaptopurine (50 mg/m²/day), intramuscular methotrexate (15 mg/m²/week) and ATRA (45 mg/m²/day) for 15 days every three months for two years (B).⁹⁸

RC was attained in 84% of patients (95% CI: 77-91) with the worst responses rates being observed in patients with platelet counts < 40 \times 10⁹/L (77% vs. 93%; p-value = 0.076) and in those older than 70 years of age (74% vs. 89%; p-value = 0.096) (B).⁹⁸

Mortality rate of 16.34% was observed during the induction treatment with deaths due to infection, pulmonary or cerebral hemorrhage and Differentiation syndrome; 3.8% of the deaths during consolidation treatment were by lung aspergillosis, cardiac dysfunction, brain hemorrhage or pulmonary infection and 2.8% of deaths during maintenance treatment were due to unrelated accidental trauma or other unknown causes. After a mean follow-up of 36 months, the cumulative rate of relapse at six years was estimated at 8.5% with 11.2% in patients aged 60 to 70 years and 0% in those over 70 years of age (p-value = 0.19). For patients who attained CR, the estimations of DFS and leukemia-free survival at six years were 79% \pm 10% and 91% \pm 8%, respectively (B).⁹⁸

In the evaluation of over 60-year-old patients with again APL treated with ATRA (45 mg/m²/day) until CR or a maximum of 90 days and systemic chemotherapy with daunorubicin (60 mg/m²/day) for three days and cytarabine (200 mg/m²/day) for seven days, the CR rate was 86%. Of the patients in CR, 18.6% died mainly due to sepsis during the consolidation cycle which consisted in daunorubicin (45 mg/m²/day) for three days and cytarabine (1 g/m² b.i.d.) for four days or during maintenance treatment with ATRA, 6MP and methotrexate. The incidence of relapse and the EFS and OS rates at four years were 15.6%, 53% and 57.8%, respectively (B).⁹⁹

To decrease the toxicity of APL treatment in elderly patients, response was assessed after induction with ATRA (45 mg/m²/day) associated with idarubicin (12 mg/m²/day) on D2, D4, D6 and D8 (as in young patients), followed by a single cycle of consolidation with idarubicin (5 mg/m²) and cytarabine (1 g/m²) on D1 to D4, and maintenance with ATRA (45 mg/m²/day) for 15 days every three months. The hematological remission

rate was 90% with 10% of deaths during induction treatment and 6.6% after remission due to bleeding or infection before or during consolidation; 18.3% relapsed after a median time of 17.5 months of CR. OS, DFS and the cumulative incidence of relapse at five years were 76.1%, 64.6% and 27.4%, respectively (B).¹⁰⁰

Newly diagnosed patients with APL, confirmed by cytogenetics or molecular study, aged 60 years or older with normal liver and kidney function and without severe heart disease or infectious disease at diagnosis were assessed as to the response to induction treatment with one or two systemic chemotherapy cycles associated with ATRA (45 mg/m²/day) until CR or for a maximum of 60 days (B).¹⁰¹

The first cycle comprised oral 6-thioguanine (100 mg/m² b.i.d. from D3 to D8), a continuous infusion of cytarabine (100 mg/m²/day on D1 and D2 followed by 100 mg/m² b.i.d. on D3 to D8) and daunorubicin (60 mg/m²/day from D3 to D5). For cases that did not obtain remission after induction, a second induction cycle was conducted with cytarabine (1 g/m² b.i.d. from D1 to D3) associated with mitoxantrone (10 mg/m² from D1 to D3) (B).¹⁰¹

RC was attained in 82% of patients with early death in 18% within two to 19 days after beginning induction therapy. The mortality rate was higher for patients with leukocyte counts > 10 \times 10⁹/L (p-value = 0.009) and in over 70-year-old patients (p-value = 0.048). The main causes of death were bleeding, multiple organ failure and sepsis (B).¹⁰¹

Recommendations

There is no evidence on which is the best treatment for APL in the elderly. Treatment similar to those conducted in patients younger than 60 years are recommended but lower response to treatment should be expected. The use of cytarabine shows no significant benefits in this population. Patients who are not eligible for treatment have short survival.

How should minimal residual disease in acute promyelocytic leukemia be monitored?

P – Acute promyelocytic leukemia

I – Polymerase chain reaction

O – Minimal residual disease

The investigation of the PML-RAR α rearrangement in patients with APL in hematologic remission (investigation of MRD) is commonly performed by RT-PCR or RQ-PCR. Diverio et al. analyzed bone marrow samples harvested at different times from 163 patients with APL treated with ATRA and anthracyclines. Of the 21 patients who converted from a negative to a positive result using RT-PCR, 20 suffered hematologic relapse after a median time of three months (range: 1-14 months). Of the 142 patients who persisted with negative results in RT-PCR tests, eight suffered hematologic relapse and 134 remained in CR (B).¹⁰²

In a group of APL patients (27.2% low-risk and 72.8% high-risk for relapse) initially treated with As₂O₃, RT-PCR and RQ-PCR were performed at diagnosis, at the end of induction

treatment, at the beginning of the consolidation phase and every three months during two years of maintenance treatment with subsequent evaluation of PML-RAR α transcripts to assess MRD and the predictive effect on relapse of the disease (B).⁷⁶ The results were positive for the PML-RAR α transcript in 100% of cases for both RT-PCR and RQ-PCR at diagnosis, 63.82% and 85.18%, respectively at the end of the induction treatment (at this time 100% of patients were in complete hematological remission), 19.49% and 34.78%, respectively before the beginning of the consolidation treatment and 0% and 27.11%, respectively during maintenance treatment (B).⁷⁶

MRD monitoring in patients with APL by RQ-PCR using peripheral blood and bone marrow after each cycle of chemotherapy and every three months after the end of consolidation treatment was a strong and independent predictor of DFS [hazard ratio (HR): 17.87; 95% CI: 6.88-46.4; *p*-value = 0.0001] better than the leukocyte count (HR: 1.02; 95% CI: 1.0-1.03; *p*-value = 0.002) and a strong and independent predictor of relapse (HR: 39.94; 95% CI: 11.06-144.18; *p*-value = 0.0001) again better than the leukocyte count (HR: 1.03; 95% CI: 1.01-1.05; *p*-value = 0.002) (B).¹⁰³ The detection of PML-RAR α transcripts at the end of induction alone was not a good predictor for DFS (B).¹⁰³ The serial evaluation of bone marrow samples proved to be superior to peripheral blood samples¹⁰³ (B) and thus the European LeukemiaNet recommends the study of bone marrow every three months (B).⁸

These results were corroborated by the study by Lee et al. in which levels of MRD $\geq 1 \times 10^{-3}$ of transcripts identified by RQ-PCR at completion of the consolidation treatment compared with values of $< 1 \times 10^{-3}$ proved to be the most important predictor of relapse (85.7 ± 13.2 vs. 7.3 ± 4.1 ; *p*-value < 0.001) and DFS (14.3 ± 13.2 vs. 91.2 ± 4.3 ; *p*-value < 0.001) (B).⁷⁸

In respect to the molecular diagnosis of APL, 23% of the cases that presented with hematologic relapse were not predicted by molecular techniques (RT-PCR or RQ-PCR). In all these cases, consistent analysis had been performed four to five months before relapse (mean: 260; range: 153-386 days) (B).¹⁰⁴

Recommendations

The monitoring of MRD should be carried using RT-PCR or RQ-PCR molecular tests with serially obtained bone marrow samples. It is recommended to test at diagnosis, after consolidation and every three months during maintenance treatment for two years.

Conflicts of interest

The authors declare no conflicts interest.

REFERENCES

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC: Lyon 2008.

2. Rohr SS, Pelloso LA, Borgo A, De Nadai LC, Yamamoto M, Rego EM, Chauffaille ML. Acute promyelocytic leukemia associated with the PLZF-RARA fusion gene: two additional cases with clinical and laboratorial peculiar presentations. *Med Oncol*. 2012;29(4):2345-7.
3. De Lourdes Chauffaille M, Borri D, Proto-Siqueira R, Moreira ES, Alberto FL. Acute promyelocytic leukemia with t(15;17): frequency of additional clonal chromosome abnormalities and FLT3. *Leuk Lymphoma*. 2008, 49 (12):2387-9.
4. Gupta SK, Sazawal S, Mahapatra M, Saxena R. Evaluation of PG-M3 antibody in the diagnosis of acute promyelocytic leukaemia. *Eur J Clin Invest*. 2010;40(10):960-2.
5. Dimov ND, Medeiros LJ, Kantarjian HM, Cortes JE, Chang KS, Bueso-Ramos CE, et al. Rapid and reliable confirmation of acute promyelocytic leukemia by immunofluorescence staining with an antipromyelocytic leukemia antibody: the M.D. Anderson Cancer Center experience of 349 patients. *Cancer*. 2010;116(2):369-76. Comment in: *Cancer*. 2011;117(2):435; author reply 435-6. Epub 2010 Sep 15.
6. Brockman SR, Paternoster SF, Ketterling RP, Dewald GW. New highly sensitive fluorescence in situ hybridization method to detect PML/RARA fusion in acute promyelocytic leukemia. *Cancer Genet Cytogenet*. 2003;145(2):144-51.
7. O'Connor SJ, Forsyth PD, Dalal S, Evans PA, Short MA, Shiach C, et al. The rapid diagnosis of acute promyelocytic leukaemia using PML (5E10) monoclonal antibody. *Br J Haematol*. 1997;99(3):597-604.
8. Sanz MA, Grimwade D, Tallman MS, Lowenberg B, Fenaux P, Estey EH, et al. Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2009;113(9):1875-91.
9. Tallman MS, Altman JK. How I treat acute promyelocytic leukemia. *Blood*. 2009;114(25):5126-35.
10. Chang H, Kuo MC, Shih LY, Dunn P, Wang PN, Wu JH, et al. Clinical bleeding events and laboratory coagulation profiles in acute promyelocytic leukemia. *Eur J Haematol*. 2012 Apr;88(4):321-8.
11. Kim DY, Lee JH, Lee JH, Kim SD, Lim SN, Choi Y, et al. Significance of fibrinogen, D-dimer, and LDH levels in predicting the risk of bleeding in patients with acute promyelocytic leukemia. *Leuk Res*. 2011;35(2):152-8.
12. Yanada M, Matsushita T, Asou N, Kishimoto Y, Tsuzuki M, Maeda Y, et al. Severe hemorrhagic complications during remission induction therapy for acute promyelocytic leukemia: incidence, risk factors, and influence on outcome. *Eur J Haematol*. 2007;78(3):213-9.
13. Dally N, Hoffman R, Haddad N, Sarig G, Rowe JM, Brenner B. Predictive factors of bleeding and thrombosis during induction therapy in acute promyelocytic leukemia-a single center experience in 34 patients. *Thromb Res*. 2005;116(2):109-14.
14. Higuchi T, Shimizu T, Mori H, Niikura H, Omine M. Coagulation patterns of disseminated intravascular coagulation in acute promyelocytic leukemia. *Hematol Oncol*. 1997;15(4):209-17.
15. Sanz MA, Lo Coco F, Martín G, Avvisati G, Rayón C, Barbui T, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic

- leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. *Blood*. 2000;96(4):1247-53.
16. Huang H, Qin Y, Xu R, You X, Teng R, Yang L, et al. Combination therapy with arsenic trioxide, all-trans retinoic acid, and chemotherapy in acute promyelocytic leukemia patients with various relapse risks. *Leuk Res*. 2012 Jul;36(7):841-5.
 17. Candoni A, Damiani D, Michelutti A, Masolini P, Michieli M, Michelutti T, et al. Clinical characteristics, prognostic factors and multidrug-resistance related protein expression in 36 adult patients with acute promyelocytic leukemia. *Eur J Haematol*. 2003;71(1):1-8.
 18. Sanz MA, Montesinos P, Vellenga E, Rayón C, de la Serna J, Parody R, et al. Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline monotherapy: long-term outcome of the LPA99 multicenter study by the PETHEMA Group. *Blood*. 2008;112(8):3130-4.
 19. Kawai Y, Watanabe K, Kizaki M, Murata M, Kamata T, Uchida H, et al. Rapid improvement of coagulopathy by all-trans retinoic acid in acute promyelocytic leukemia. *Am J Hematol*. 1994;46(3):184-8.
 20. Rego EM, Kim HT, Ruiz-Argüelles GJ, Undurraga MS, Uriarte Mdel R, Jacomo RH, et al. Improving acute promyelocytic leukemia (APL) outcome in developing countries through networking, results of the International Consortium on APL. *Blood*. 2013;121(11):1935-43. Comment in: *Blood*. 2013;121(11):1925-6.
 21. Drapkin RL, Gee TS, Dowling MD, Arlin Z, McKenzie S, Kempin S, et al. Prophylactic heparin therapy in acute promyelocytic leukemia. *Cancer*. 1978;41(6):2484-90.
 22. Tallman MS, Kwaan HC. Reassessing the hemostatic disorder associated with acute promyelocytic leukemia. *Blood*. 1992;79(3):543-53. Comment in: *Blood*. 1992;80(5):1365-6.
 23. Zhao W, Wang X, Guo W, Qu B, Wang H, Shen Z, et al. Hemostatic abnormalities associated with acute promyelocytic leukemia and corrective effects of all-trans-retinoic acid or arsenic trioxide treatment. *Chin Med J (Engl)*. 2000;113(3):236-40.
 24. Escudier SM, Kantarjian HM, Estey EH. Thrombosis in patients with acute promyelocytic leukemia treated with and without all-trans retinoic acid. *Leuk Lymphoma*. 1996;20(5-6):435-9.
 25. Goldberg MA, Ginsburg D, Mayer RJ, Stone RM, Maguire M, Rosenthal DS, et al. Is heparin administration necessary during induction chemotherapy for patients with acute promyelocytic leukemia? *Blood*. 1987;69(1):187-91.
 26. Hoyle CF, Swirsky DM, Freedman L, Hayhoe FG. Beneficial effect of heparin in the management of patients with APL. *Br J Haematol*. 1988;68(3):283-9.
 27. Bernard J, Weil M, Boiron M, Jacquillat C, Flandrin G, Gemon MF. Acute promyelocytic leukemia: results of treatment with daunorubicin. *Blood*. 1973;41(4):489-96.
 28. Lengfelder E, Hanfstein B, Haferlach C, Braess J, Krug U, Spiekermann K, et al. Outcome of elderly patients with acute promyelocytic leukemia: results of the German Acute Myeloid Leukemia Cooperative Group. *Ann Hematol*. 2013;92(1):41-52.
 29. Iland H, Bradstock K, Seymour J, Hertzberg M, Grigg A, Taylor K, et al. Results of the APML3 trial incorporating all-trans-retinoic acid and idarubicin in both induction and consolidation as initial therapy for patients with acute promyelocytic leukemia. *Haematologica*. 2012;97(2):227-34.
 30. Avvisati G, Lo-Coco F, Paoloni FP, Petti MC, Diverio D, Vignetti M, et al. AIDA 0493 protocol for newly diagnosed acute promyelocytic leukemia: very long-term results and role of maintenance. *Blood*. 2011;117(18):4716-25.
 31. Adès L, Guerri A, Raffoux E, Sanz M, Chevalier P, Lapusan S et al. Very long-term outcome of acute promyelocytic leukemia after treatment with all-trans-retinoic acid and chemotherapy: the European Group APL experience. *Blood*. 2010;115(9):1690-6.
 32. Pagnano KB, de Carvalho Duarte G, Lorand-Metze I, Delamain MT, Miranda EC, De Souza CA. Treatment outcome of acute promyelocytic leukemia with modified aida protocol. *Adv Hematol*. 2010;2010:672137.
 33. Shen ZX, Shi ZZ, Fang J, Gu BW, Li JM, Zhu YM, et al. All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci U S A*. 2004;101(15):5328-35.
 34. Wang H, Chen XY, Wang BS, Rong ZX, Qi H, Chen HZ. The efficacy and safety of arsenic trioxide with or without all-trans retinoic acid for the treatment of acute promyelocytic leukemia: a meta-analysis. *Leuk Res*. 2011;35(9):1170-7.
 35. Elemam O, Abdelmoety D. Acute promyelocytic leukemia, study of predictive factors for Differentiation Syndrome, single center experience. *J Egypt Natl Canc Inst*. 2013;25(1):13-9.
 36. Breccia M, Mazzarella L, Bagnardi V, Disalvatore D, Loggisci G, Cimino G, et al. Increased BMI correlates with higher risk of disease relapse and differentiation syndrome in patients with acute promyelocytic leukemia treated with the AIDA protocols. *Blood*. 2012;119(1):49-54.
 37. De Botton S, Dombret H, Sanz M, Miguel JS, Caillot D, Zittoun R, et al. Incidence, clinical features, and outcome of all trans-retinoic acid syndrome in 413 cases of newly diagnosed acute promyelocytic leukemia. The European APL Group. *Blood*. 1998;92(8):2712-8.
 38. Su YC, Dunn P, Shih LY, Kuo MC, Chang H, Wu JH, et al. Retinoic acid syndrome in patients following the treatment of acute promyelocytic leukemia with all-trans retinoic acid. *Chang Gung Med J*. 2009;32(5):535-42.
 39. Jeddi R, Ghédira H, Mnif S, Gouider E, Fenaux P, Meddeb B. High body mass index is an independent predictor of differentiation syndrome in patients with acute promyelocytic leukemia. *Leuk Res*. 2010;34(4):545-7.
 40. Montesinos P, Bergua JM, Vellenga E, Rayón C, Parody R, de la Serna J, León A, Esteve J, Milone G, Debén G, Rivas C, González M, Tormo M, Díaz-Mediavilla J, González JD, Negri S, Amutio E, Brunet S, Lowenberg B, Sanz MA. Differentiation syndrome in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline chemotherapy: characteristics, outcome, and prognostic factors. *Blood*. 2009;113(4):775-83.
 41. Jeddi R, Kacem K, Ben Neji H, Mnif S, Gouider E, Aissaoui L, et al. Predictive factors of all-trans-retinoic acid

- related complications during induction therapy for acute promyelocytic leukemia. *Hematology*. 2008;13(3):142-6.
42. Montesinos P, Sanz M. The differentiation syndrome in patients with acute promyelocytic leukemia: experience of pethema group and review of the literature. *Mediterr J Hematol Infect Dis*. 2011;3(1):e2011059.
 43. Sastre A, Gago E, Baños M, Gómez E. Acute renal failure in the transretinóico syndrome. *Nefrologia*. 2007;27(2):184-90.
 44. Vahdat L, Maslak P, Miller WH Jr, Eardley A, Heller G, Scheinberg DA, et al. Early mortality and the the retinoic acid syndrome in acute promyelocytic leukemia: impact of leukocytosis, low dose-dose chemotherapy, PMN/RAR-alpha isoform, and CD13 expression in patient treated with all-trans retinoic acid. *Blood*. 1994;84(11):3843-9.
 45. Wiley JS, Firkin FC. Reduction of pulmonary toxicity by prednisolone prophylaxis during all-trans retinoic acid treatment of acute promyelocytic leukemia. Australian Leukemia Study Group. *Leukemia*. 1995;9(5):774-8.
 46. Sanz MA, Montesinos P, Rayón C, Holowiecka A, de la Serna J, Milone G, et al. Risk-adapted treatment of acute promyelocytic leukemia based on all-trans retinoic acid and anthracycline with addition of cytarabine in consolidation therapy for high-risk patients: further improvements in treatment outcome. *Blood*. 2010;115(25):5137-46. Comment in: *Nat Rev Clin Oncol*. 2010;7(9):484.
 47. de la Serna J, Montesinos P, Vellenga E, Rayón C, Parody R, León A, et al. Causes e prognostic factors of remission induction failure in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and idarubicin. *Blood*. 2008;111(7):3395-402.
 48. Tiamkao S, Sirijirachai C. Pseudotumor cerebri caused by all-trans-retinoic acid: a case report. *J Med Assoc Thai*. 2000;83(11):1420-3.
 49. Mahmoud HH, Hurwitz CA, Roberts WM, Santana VM, Ribeiro RC, Krance RA. Tretinoin toxicity in children with acute promyelocytic leukaemia. *Lancet*. 1993;342(8884):1394-5.
 50. Selleri C, Pane F, Notaro R, Catalano L, Santoro LE, Luciano L, et al. All-trans-retinoic acid (ATRA) responsive skin relapses of acute promyelocytic leukaemia followed by ATRA-induced pseudotumour cerebri. *Br J Haematol*. 1996;92(4):937-40.
 51. Visani G, Bontempo G, Manfroi S, Pazzaglia A, D'Alessandro R, Tura S. All-trans-retinoic acid and pseudotumor cerebri in a young adult with acute promyelocytic leukemia: a possible disease association. *Haematologica*. 1996;81(2):152-4.
 52. Chen HY, Tsai RK, Huang SM. ATRA-induced pseudotumour cerebri--one case report. *Kaohsiung J Med Sci*. 1998;14(1):58-60.
 53. Sano F, Tsuji K, Kunika N, Takeuchi T, Oyama K, Hasegawa S, et al. Pseudotumor cerebri in a patient with acute promyelocytic leukemia during treatment with all-trans retinoic acid. *Intern Med*. 1998;37(6):546-9.
 54. Naderi S, Nukala S, Marruenda F, Kudarvalli P, Koduri PR. Pseudotumour cerebri in acute promyelocytic leukemia: improvement despite continued ATRA therapy. *Ann Hematol*. 1999;78(7):333-4.
 55. Galm O, Fabry U, Osieka R. Pseudotumor cerebri after treatment of relapsed acute promyelocytic leukemia with arsenic trioxide. *Leukemia*. 2000;14(2):343-4.
 56. Colucciello M. Pseudotumor cerebri induced by all-trans retinoic acid treatment of acute promyelocytic leukemia. *Arch Ophthalmol*. 2003;121(7):1064-5.
 57. Yeh YC, Tang HF, Fang IM. Pseudotumor cerebri caused by all-trans-retinoic acid treatment for acute promyelocytic leukemia. *Jpn J Ophthalmol*. 2006;50(3):295-9.
 58. Dixon KS, Hassoun A. Pseudotumor cerebri due to the potentiation of all-trans retinoic acid by voriconazole. *J Am Pharm Assoc* (2003). 2010;50(6):742-4.
 59. Machner B, Neppert B, Paulsen M, Hofmann C, Sander T, Helmchen C. Pseudotumor cerebri as a reversible side effect of all-trans retinoic acid treatment in acute promyelocytic leukaemia. *Eur J Neurol*. 2008;15(7):e68-9.
 60. Gallipoli P, Drummond MW. Pseudotumour cerebri as a manageable side effect of prolonged all-trans retinoic acid therapy in an adult patient with acute promyelocytic leukaemia. *Eur J Haematol*. 2009;82(3):242-3. Erratum in: *Eur J Haematol*. 2009;82(5):411. Drummond, M W [added].
 61. Schroeter T, Lanvers C, Herding H, Suttrop M. Pseudotumor cerebri induced by all-trans-retinoic acid in a child treated for acute promyelocytic leukemia. *Med Pediatr Oncol*. 2000;34(4):284-6.
 62. Decaudin D, Adams D, Naccache P, Castagna L, Munck JN. Maintained all-trans retinoic acid therapy in a patient with pseudotumour cerebri despite aggravated symptoms. *Leuk Lymphoma*. 1997;27(3-4):373-4.
 63. Specchia G, Lo Coco F, Vignetti M, Avvisati G, Fazi P, Albano F, et al. Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not with all-trans retinoic acid: a report by the Gruppo Italiano Malattie Ematologiche dell'Adulto. *J Clin Oncol*. 2001;19(20):4023-8.
 64. Kakimoto T, Takahashi Y, Watanabe H, Matsuzawa M, Sanada Y, et al. Primary multiple cerebral invasion in acute promyelocytic leukemia dramatically worsened by all-trans retinoic acid. *Leuk Res*. 2010;34(5):e137-9.
 65. de Botton S, Sanz MA, Chevret S, Dombret H, Martin G, Thomas X, et al. Extramedullary relapse in acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. *Leukemia*. 2006;20(1):35-41.
 66. Adès L, Sanz MA, Chevret S, et al. Treatment of newly diagnosed acute promyelocytic leukemia (APL): a comparison of French-Belgian-Swiss and PETHEMA results. *Blood*. 2008 Feb 1;111(3):1078-84. Epub 2007 Nov 1.
 67. Adès L, Chevret S, Raffoux E, de Botton S, Guerci A, Pigneux A, et al. Is cytarabine useful in the treatment of acute promyelocytic leukemia? Results of a randomized trial from the European Acute Promyelocytic Leukemia Group. *J Clin Oncol*. 2006;24(36):5703-10.
 68. Sanz MA, Vellenga E, Rayón C, Díaz-Mediavilla J, Rivas C, Amutio E, et al. All-trans retinoic acid and anthracycline monochemotherapy for the treatment of elderly patients with acute promyelocytic leukemia. *Blood*. 2004;104(12):3490-3.

69. Fenaux P, Chastang C, Chevret S, Sanz M, Dombret H, Archimbaud E, et al. A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. The European APL Group. *Blood*. 1999;94(4):1192-200.
70. Sanz MA, Montesinos P, Rayón C, Holowiecka A, de la Serna J, Milone G, et al. Risk-adapted treatment of acute promyelocytic leukemia based on all-trans retinoic acid and anthracycline with addition of cytarabine in consolidation therapy for high-risk patients: further improvements in treatment outcome. *Blood*. 2010;115(25):5137-46.
71. Schlenk RF, Germing U, Hartmann F, Glasmacher A, Fischer JT, del Valle y Fuentes F, et al. High-dose cytarabine and mitoxantrone in consolidation therapy for acute promyelocytic leukemia. *Leukemia*. 2005;19(6):978-83.
72. de Botton S, Fawaz A, Chevret S, Dombret H, Thomas X, Sanz M, et al. Autologous and allogeneic stem-cell transplantation as salvage treatment of acute promyelocytic leukemia initially treated with all-trans-retinoic acid: a retrospective analysis of the European acute promyelocytic leukemia group. *J Clin Oncol*. 2005;23(1):120-6.
73. Sanz MA, Labopin M, Gorin NC, de la Rubia J, Arcese W, Meloni G, et al. Hematopoietic stem cell transplantation for adults with acute promyelocytic leukemia in the ATRA era: a survey of the European Cooperative Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2007;39(8):461-9.
74. Dvorak CC, Agarwal R, Dahl GV, Gregory JJ, Feusner JH. Hematopoietic stem cell transplant for pediatric acute promyelocytic leukemia. *Biol Blood Marrow Transplant*. 2008;14(7):824-30. PubMed PMID: 18541203
75. Apperley J, Carreras E, Gluckman E, Masszi T. Hematopoietic Stem Cell Transplantation. The EBMT Handbook 2012.
76. Chendamarai E, Balasubramanian P, George B, Viswabandya A, Abraham A, Ahmed R, et al. Role of minimal residual disease monitoring in acute promyelocytic leukemia treated with arsenic trioxide in frontline therapy. *Blood*. 2012;119(15):3413-9.
77. Polampalli S, Choughule A, Prabhaskar K, Amare P, Baisane C, Kabre S, et al. Role of RT-PCR and FISH in diagnosis and monitoring of acute promyelocytic leukemia. *Indian J Cancer*. 2011;48(1):60-7.
78. Lee S, Kim YJ, Eom KS, Min CK, Kim HJ, Cho SG, et al. The significance of minimal residual disease kinetics in adults with newly diagnosed PML-RARalpha-positive acute promyelocytic leukemia: results of a prospective trial. *Haematologica*. 2006;91(5):671-4.
79. Liu YF, Zhu YM, Shen SH, Shen ZX, Li JM, Chen SJ, et al. Molecular response in acute promyelocytic leukemia: a direct comparison of regular and real-time RT-PCR. *Leukemia*. 2006;20(8):1393-9.
80. Santamaría C, Chillón MC, Fernández C, Martín-Jiménez P, Balanzategui A, García Sanz R, et al. Using quantification of the PML-RARalpha transcript to stratify the risk of relapse in patients with acute promyelocytic leukemia. *Haematologica*. 2007;92(3):315-22.
81. Raffoux E, Rousselot P, Poupon J, Daniel MT, Cassinat B, Delarue R, et al. Combined treatment with arsenic trioxide and all-trans-retinoic acid in patients with relapsed acute promyelocytic leukemia. *J Clin Oncol*. 2003;21(12):2326-34.
82. Carradice D, Austin N, Bayston K, Ganly PS. Successful treatment of acute promyelocytic leukaemia during pregnancy. *Clin Lab Haematol*. 2002;24(5):307-11.
83. Yang D, Hladnik L. Treatment of acute promyelocytic leukemia during pregnancy. *Pharmacotherapy*. 2009;29(6):709-24.
84. Watanabe R, Okamoto S, Moriki T, Kizaki M, Kawai Y, Ikeda Y. Treatment of acute promyelocytic leukemia with all-trans retinoic acid during the third trimester of pregnancy. *Am J Hematol*. 1995;48(3):210-1.
85. Requena A, Velasco JG, Pinilla J, Gonzalez-Gonzalez A. Acute leukemia during pregnancy: obstetric management and perinatal outcome of two cases. *Eur J Obstet Gynecol Reprod Biol*. 1995;63(2):139-41.
86. Incerpi MH, Miller DA, Posen R, Byrne JD. All-trans retinoic acid for the treatment of acute promyelocytic leukemia in pregnancy. *Obstet Gynecol*. 1997;89(5 Pt 2):826-8.
87. Giagounidis AA, Beckmann MW, Giagounidis AS, Aivado M, Emde T, Germing U, et al. Acute promyelocytic leukemia and pregnancy. *Eur J Haematol*. 2000;64(4):267-71.
88. Fadilah SA, Hatta AZ, Keng CS, Jamil MA, Singh S. Successful treatment of acute promyelocytic leukemia in pregnancy with all-trans retinoic acid. *Leukemia*. 2001;15(10):1665-6.
89. Nakamura K, Dan K, Iwakiri R, Gomi S, Nomura T. Successful treatment of acute promyelocytic leukemia in pregnancy with all-trans retinoic acid. *Ann Hematol*. 1995 Nov;71(5):263-4.
90. Lipovsky MM, Biesma DH, Christiaens GC, Petersen EJ. Successful treatment of acute promyelocytic leukaemia with all-trans-retinoic-acid during late pregnancy. *Br J Haematol*. 1996;94(4):699-701.
91. Terada Y, Shindo T, Endoh A, Watanabe M, Fukaya T, Yajima A. Fetal arrhythmia during treatment of pregnancy-associated acute promyelocytic leukemia with all-trans retinoic acid and favorable outcome. *Leukemia*. 1997;11(3):454-5.
92. Takitani K, Hino N, Terada Y, Kurosawa Y, Koh M, Inoue A, et al. Plasma all-trans retinoic acid level in neonates of mothers with acute promyelocytic leukemia. *Acta Haematol*. 2005;114(3):167-9.
93. Culligan DJ, Merriman L, Kell J, et al. The management of acute promyelocytic leucemia presenting during pregnancy. *Clin Leukemia* 2007;1:183-191.
94. Cardonick E, Iacobucci A. Use of chemotherapy during human pregnancy. *Lancet Oncol*. 2004;5(5):283-91.
95. Siu BL, Alonzo MR, Vargo TA, Fenrich AL. Transient dilated cardiomyopathy in a newborn exposed to idarubicin and all-trans retinoic acid (ATRA) early in the second trimester of pregnancy. *Int J Gynecol Cancer* 2002;12(4):399-402.
96. Tallman MS, Andersen JW, Schiffer CA, Appelbaum FR, Feusner JH, Ogden A, et al. All-trans retinoic acid in acute promyelocytic leukemia. *N Engl J Med*. 1997;337(15):1201-8. Erratum in: *N Engl J Med* 1997;337(22):1639.

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97. Latagliata R, Avvisati G, Lo Coco F, Petti MC, Diverio D, Spadea A, et al. The role of all-trans-retinoic acid (ATRA) treatment in newly-diagnosed acute promyelocytic leukemia patients aged > 60 years. *Ann Oncol.* 1997;8(12):1273-5.
98. Sanz MA, Vellenga E, Rayón C, Díaz-Mediavilla J, Rivas C, Amutio E, et al. All-trans retinoic acid and anthracycline monotherapy for the treatment of elderly patients with acute promyelocytic leukemia. *Blood.* 2004;104(12):3490-3.
99. Ades L, Chevret S, De Botton S, Thomas X, Dombret H, Beve B, et al; European APL Group. Outcome of acute promyelocytic leukemia treated with all trans retinoic acid and chemotherapy in elderly patients: the European group experience. *Leukemia.* 2005;19(2):230-3.
100. Latagliata R, Breccia M, Fazi P, Vignetti M, Di Raimondo F, Sborgia M, et al. GIMEMA AIDA 0493 amended protocol for elderly patients with acute promyelocytic leukaemia. Long-term results and prognostic factors. *Br J Haematol.* 2011;154(5):564-8.
101. Lengfelder E, Hanfstein B, Haferlach C, Braess J, Krug U, Spiekermann K, et al. Outcome of elderly patients with acute promyelocytic leukemia: results of the German Acute Myeloid Leukemia Cooperative Group. *Ann Hematol.* 2013;92(1):41-52.
102. Diverio D, Rossi V, Avvisati G, De Santis S, Pistilli A, Pane F, et al. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARalpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. *Blood.* 1998;92(3):784-9.
103. Santamaría C, Chillón MC, Fernández C, Martín-Jiménez P, Balanzategui A, García Sanz R, et al. Using quantification of the PML-RARalpha transcript to stratify the risk of relapse in patients with acute promyelocytic leukemia. *Haematologica.* 2007;92(3):315-22.
104. Grimwade D, Jovanovic JV, Hills RK, Nugent EA, Patel Y, Flora R, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. *J Clin Oncol.* 2009;27(22):3650-8.