ORIGINAL ARTICLE

Analysis of factors affecting hemorrhagic diathesis and overall survival in patients with acute promyelocytic leukemia

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Background/Aims: This study investigated whether patients with acute promyelocytic leukemia (APL) truly fulfill the diagnostic criteria of overt disseminated intravascular coagulation (DIC), as proposed by the International Society on Thrombosis and Haemostasis (ISTH) and the Korean Society on Thrombosis and Hemostasis (KSTH), and analyzed which component of the criteria most contributes to bleeding diathesis.

Methods: A single-center retrospective analysis was conducted on newly diagnosed APL patients between January 1995 and May 2012.

Results: A total of 46 newly diagnosed APL patients were analyzed. Of these, 27 patients (58.7%) showed initial bleeding. The median number of points per patient fulfilling the diagnostic criteria of overt DIC by the ISTH and the KSTH was 5 (range, 1 to 7) and 3 (range, 1 to 4), respectively. At diagnosis of APL, 22 patients (47.8%) fulfilled the overt DIC diagnostic criteria by either the ISTH or KSTH. In multivariate analysis of the ISTH or KSTH diagnostic criteria for overt DIC, the initial fibrinogen level was the only statistically significant factor associated with initial bleeding (p = 0.035), but it was not associated with overall survival (OS).

Conclusions: Initial fibrinogen level is associated with initial presentation of bleeding of APL patients, but does not affect OS.

Keywords: Leukemia, promyelocytic, acute; Disseminated intravascular coagulation; Fibrinogen

INTRODUCTION

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML). It is classified as an aggressive form of AML with the chromosomal translocation t(15;17)(q22;q12) occurring in myeloid cells, according to the World Health Organization classification [1]. This balanced translocation results in fusion between the retinoic acid receptor α gene (*RARA*) on chromosome 17q12 and a nuclear regulatory factor gene

(promyelocytic leukemia or *PML* gene) on chromosome 15. The *PML-RARA* fusion gene produces a chimeric protein that arrests maturation of myeloid cells at the promyelocytic stage [2].

The current standards of induction therapy with simultaneous all-trans retinoic acid (ATRA) and anthracycline-based chemotherapy yield a complete remission rate of 90% to 95% [3], and the cure rate of APL is approximately 80% to 90% [4].

Bleeding in patients with APL can appear in various

forms, such as widespread bruising, petechiae, mucus membrane bleeding, central nervous system bleeding, pulmonary hemorrhage, gastrointestinal hemorrhage, and excessive blood loss from sites of trauma [5]. Coagulopathy causing such bleeding is life threatening, and is the leading cause of death for patients with APL. An approximately 10% early death rate has been reported in cooperative group clinical trials; however, it appears to be nearly twice as high in population-based studies [6]. A retrospective analysis of 134 Brazilian patients with APL reported a death rate of 32% during induction, with the majority of deaths (60.5%) due to hemorrhage [7]. Therefore, immediate treatment with ATRA should be initiated in suspected APL cases, even before a definitive diagnosis can be made. After administration of ATRA, APL has a high cure rate and coagulopathy typically improves after 5 to 7 days of treatment [8].

Similar to classical disseminated intravascular coagulation (DIC), APL-associated coagulopathy is characterized by activation of a coagulation cascade leading to thrombus formation, hypoperfusion, and bleeding due to widespread consumption of platelets and clotting factors [5]. In addition, fibrinolysis occurs secondary to DIC due to the release of proteolytic enzyme granules from APL blasts, resulting in thrombosis, hyperfibrinolysis, and coagulopathy [9].

The International Society on Thrombosis and Haemostasis (ISTH) and the Korean Society on Thrombosis and Hemostasis (KSTH) DIC scoring system provides objective measurement of DIC [10]. The concordance rate between the two diagnostic systems is 84.7%. When DIC occurs, the scoring system correlates with key clinical observations and outcomes [11]. However, overt DIC criteria have not been established for APL patients. Although bleeding diathesis is not always solely related to DIC, the diagnostic criteria of overt DIC are used to determine which patients have bleeding tendencies that can be prevented by supportive care.

This study investigated whether Korean APL patients truly fulfill the diagnostic criteria of overt DIC proposed by the ISTH and the KSTH, and analyzed which component of the criteria most contributes to bleeding diathesis.

METHODS

Patients and samples

A retrospective analysis was conducted on 46 newly diagnosed APL patients at Dong-A University Medical Center in Busan, South Korea, between January 1995 and May 2012. All of the patients were treated with ATRA alone or ATRA plus anthracycline for induction therapy. The study was approved by the Dong-A University Medical Center Institutional Review Board.

Diagnosis of APL

AML was diagnosed based on bone marrow biopsy and findings of aspiration, flow cytometry, cytogenetic analyses, and molecular genetics analyses. A blast count of 20% from bone marrow aspirate or peripheral blood was diagnostic for AML. Cell surface markers identified by flow cytometry included CD13, CD33, and/or CD34, which are found on normal immature myeloid cells. We also routinely tested for specific cytogenetic and molecular genetic abnormalities. APL was diagnosed when APL morphology was observed, and the presence of t(15;17) or the *PML-RARA* hybrid gene was confirmed by cytogenetic or molecular analysis, respectively.

DIC score

DIC scores of patients were calculated based on both the ISTH and KSTH scoring systems (Table 1).

Statistical analysis

Patient characteristics were summarized using descriptive statistics. The association between each diagnostic criterion or other categorical variables and the initial bleeding was analyzed by Student t test or chi-square test, respectively. Logistic regression was used to analyze the factors associated with initial bleeding. Survival analyses were performed using Kaplan-Meier estimate and log-rank tests. The Cox proportional hazards regression model was also employed in both univariate and multivariate analyses for overall survival (OS). p values less than 0.05 were considered statistically significant. All of the statistical tests were performed using SPSS version 20.0 (IBM Co., Armonk, NY, USA).

Table 1. Diagnostic criteria for overt DIC by the ISTH and the KSTH

Variable	Overt DIC by ISTH	Overt DIC by KSTH
Platelet count	50,000–100,000/μL: 1 point < 50,000/μL: 2 points	< 100,000/μL: 1 point
PT/aPTT	Prolongation of PT 3–6 sec: 1 point > 6 sec: 2 points	Prolongation of PT > 3 sec: 1 point or prolongation of aPTT > 5 sec: 1 point
Fibrinogen	< 100 mg/dL: 1 point	< 150 mg/dL: 1 point
D-dimer	0.5–1 μg/mL: 1 point 1–2 μg/mL: 2 points ≥ 2 μg/mL: 3 points	Increase: 1 point
Total	Overt DIC ≥ 5 points	Overt DIC ≥ 3 points

Adapted from Lee et al. [10].
DIC, disseminated intravascular coagulation; ISTH, International Society on Thrombosis and Haemostasis; KSTH, Korean Society on Thrombosis and Hemostasis; PT, prothrombin time; aPTT, activated partial thromboplastin time.

RESULTS

A total of 46 newly diagnosed APL patients were analyzed. The baseline characteristics of the 46 patients organized by initial bleeding manifestation are listed in Table 2. The median age was 46 years (range, 19 to 73) including eight patients older than 60 years (17.4%), and the male to female ratio was 21:25. The median white blood cell (WBC) count was 2.1×10^9/L (range, 0.28 to 137.77), the median hemoglobin level was 8.3 g/dL (range, 3.9 to 13.8), and median platelet count was 27.5×10^9/L (range, 5 to 110). There were 14 patients at low risk (WBC ≤ 10×10^9/L, platelets > 40×10^9/L, 30.4%), 23 patients at intermediate risk (WBC ≤ 10×10^9/L, platelets ≤ 40×10^9/L, 50.0%), and nine patients at high risk (WBC > 10×10^9/L, 19.6%) on the basis of risk stratification suggested by the Programa de Estudio y Tratamiento de las Hemopatias Malignas (PETHEMA) [12].

There were 27 patients (58.7%) who exhibited initial bleeding. Gum bleeding was the most common manifestation (nine cases), followed by petechiae or easy bruising (eight cases), vaginal bleeding (seven cases), epistaxis (two cases), and melena (one case). The median number of points per patient fulfilling the diagnostic criteria of overt DIC by the ISTH and the KSTH was 5 (range, 1 to 7) and 3 (range, 1 to 4), respectively. In total, 22 patients (47.8%) fulfilled the overt DIC diagnostic criteria of either the ISTH or the KSTH at the diagnosis of APL. Fulfilling the diagnostic criteria of overt DIC

by the KSTH was significantly associated with bleeding at initial presentation ($p = 0.008$). Multivariate analysis revealed that fibrinogen level was the only statistically significant factor associated with initial bleeding ($p = 0.035$) (Table 3). Early hemorrhagic death (within the first 14 days of treatment) occurred in six patients (6/46, 13%) due to fatal bleeding, including four patients with intracranial hemorrhage and two patients with pulmonary hemorrhage. The mortality rate during remission induction treatment (including willing cessation of treatment) was 23.9% (11/46). Causes of death other than fatal bleeding included sepsis (three cases), uncontrolled ATRA syndrome (one case), and unknown cause due to early willing discharge (one case).

The median follow-up duration was 22.6 months, and the median OS of analyzed patients was 122.6 months. The 2- and 5-year survival rates were 69.1% and 60.8%, respectively (Fig. 1). Univariate and multivariate analyses revealed that the factors making up the diagnostic criteria for the ISTH and KSTH of overt DIC did not significantly affect OS. There were no differences in OS between patients that fulfilled the diagnostic criteria of overt DIC (by either the ISTH or KSTH) and those without overt DIC ($p = 0.188$ or $p = 0.334$, respectively). There were no differences in OS according to initial bleeding ($p = 0.102$) (Fig. 2). In addition, initial fibrinogen level grouped by the ISTH criterion (< 100 or ≥ 100 mg/dL) and by the KSTH criterion (< 150 or ≥ 150 mg/dL) did not affect OS ($p = 0.177$ and $p = 0.334$, respectively). Interest-

Table 2. Baseline characteristics between initial bleeding group and no initial bleeding group

Characteristic	Initial bleeding (n = 27)	No initial bleeding (n = 19)	p value
ISTH criteria			0.825
Satisfied	14 (51.9)	8 (42.1)	
Unsatisfied	11 (40.7)	9 (47.4)	
Missed	2 (7.4)	2 (10.5)	
KSTH criteria			0.008
Satisfied	18 (66.7)	4 (21.1)	
Unsatisfied	7 (25.9)	13 (68.4)	
Missed	2 (7.4)	2 (10.5)	
PETHEMA risk			0.408
High	4 (14.8)	5 (26.4)	
Intermediate	16 (59.3)	7 (36.8)	
Low	7 (25.9)	7 (36.8)	
Sex			0.063
Female	17 (62.9)	8 (42.1)	
Male	10 (37.1)	11 (57.9)	
Age, yr	46 (19–65)	46 (24–73)	0.442
White blood cell, × 10⁹/L	2.0 (0.28–137.77)	1.93 (0.28–107.60)	0.678
Hemoglobin, g/dL	8.8 (4.5–13.8)	8.2 (3.9–11.4)	0.404
Platelet, × 10⁹/L	19.0 (0.5–110.0)	40.0 (0.9–80.0)	0.224
Prothrombin time, sec	14.2 (10.7–24.0)	13.0 (11.2–17.5)	0.054
aPTT, sec	25.3 (19.9–45.8)	25.9 (20.3–33.5)	0.751
Fibrinogen, mg/dL	111.4 (39.6–305.9)	181.0 (92.5–488.0)	0.002
Fibrin degradation product, mg/mL	40 (19.7–105.4)	40 (0.3–117.4)	0.594
D-dimer, µg/mL	16 (5–7,230)	19.47 (1.7–3,572.0)	0.963

Values are presented as number (%) or median (range).
ISTH, International Society on Thrombosis and Haemostasis; KSTH, Korean Society on Thrombosis and Hemostasis; PET-HEMA, Programa de Estudio y Tratamiento de las Hemopatias Malignas; aPTT, activated partial thromboplastin time.

Table 3. Multivariate analysis for factors associated with initial bleeding

Variable	OR (95% CI)	p value
Sex	0.428 (0.096–1.908)	0.266
Prothrombin time, sec	1.079 (0.774–1.504)	0.654
Fibrinogen	0.990 (0.982–0.999)	0.035

OR, odds ratio; 95% CI; 95% confidence interval.

ingly, OS was significantly improved after 2005. Before 2005, the median survival time was 26.6 months, with a 2-year and 5-year survival rate of 52.8% and 39.6%, respectively. After 2005, the median survival time was 122.6 months, with 2- and 5-year survival rates of 84.2% and 84.2%, respectively (p = 0.03) (Fig. 3).

DISCUSSION

APL differs from other subtypes of AML in that it typically presents with a life-threatening hemorrhagic diathesis. The clinical and laboratory features of coagulopathy are useful for diagnosis of DIC, and approximately
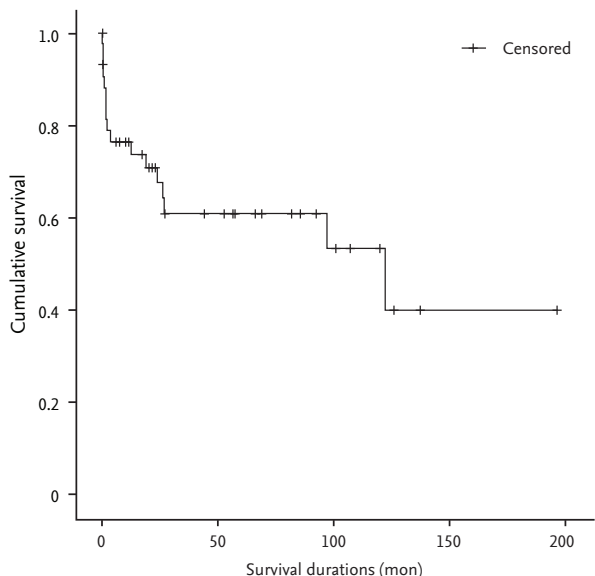
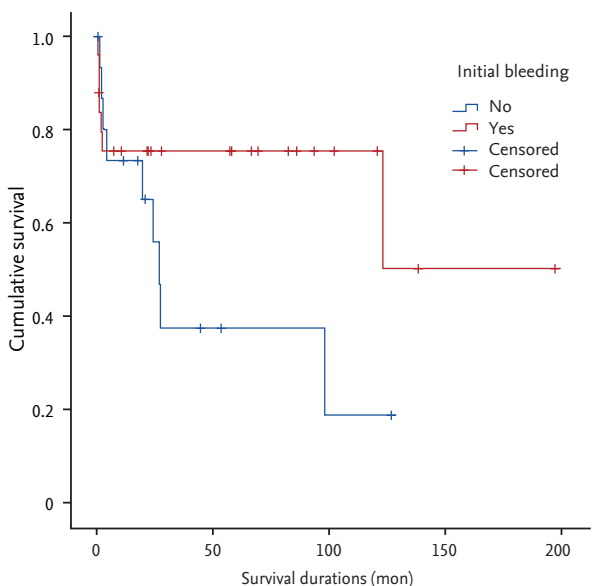**Figure 1.** Overall survival curve of all 46 analyzed patients.

Figure 2. Overall survival (OS) curve according to the presence of initial bleeding. There were no statistically significant differences in OS between the initial bleeding and no initial bleeding groups (p = 0.102).

Figure 3. Overall survival (OS) according to the year of diagnosis (before and after 2005). OS was significantly improved after 2005 (p = 0.03). The number of patients who were diagnosed before and after 2005 was 26 and 20 cases, respectively.

65% to 90% of patients have DIC around the time of diagnosis [8,12]. However, there have been no reports on whether the laboratory results of APL patients at initial diagnosis fulfill the diagnostic criteria of 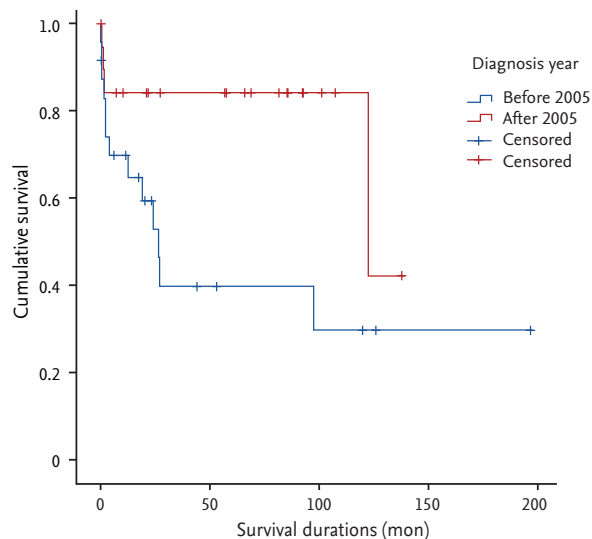overt DIC proposed by the ISTH in 2001. The KSTH proposed diagnostic criteria for overt DIC and the high agreement between the ISTH and the KSTH criteria was reported [10]. Only 47.8% of the patients in this study fulfilled the diagnostic criteria of overt DIC by either the ISTH or KSTH. There were no cases of thrombosis, and the only factor significantly associated with initial bleeding was the initial fibrinogen level. These findings suggest that coagulopathy of APL expressed as DIC may require caution, and prospective studies are needed. The consensual definition of DIC proposed by the ISTH is as follows: "DIC is an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction" [13]. In APL, coagulopathy triggered by release of proteolytic enzyme granules from APL blasts not only damages the organ microvasculature but also induces bleeding in APL patients. Since the early 1970s, clotting abnormalities of APL have been ascribed to DIC; thus, it seemed logical to propose heparin to control intravascular clotting with subsequent use of hemostatic factors [12]. However, the beneficial effects of heparin or antifibrinolytic agents have never been proven by prospective randomized trials. According to the PETHEMA leucemia promielocitica aguda (LPA) 99 trial, use of systemic

tranexamic acid for the prevention of hemorrhage did not decrease hemorrhagic mortality. However, there was a trend towards a higher incidence of thrombosis [14]. Thrombotic complications, in many cases fatal, have also been reported. However, as these are less well-recognized features of APL their incidence may be underestimated [9].

In this study, multivariate analysis revealed that initial fibrinogen level was the only factor associated with initial bleeding. These findings suggest that initial bleeding in APL patients may not be caused by overt DIC. However, fulfillment of the overt DIC diagnostic criteria may help to predict bleeding tendency and the need for more aggressive prophylaxis for bleeding in APL patients with fibrinogen less than 150 mg/dL, even without hemorrhage at presentation. Generally, platelets, fresh frozen plasma, and cryoprecipitate transfusions are needed to manage APL-associated coagulopathy. The results of this study suggest that maintaining a sufficient fibrinogen levels is just as important as maintaining platelet levels.

Hemorrhagic complications are associated with high rates of morbidity and are the leading cause of death in APL, particularly at presentation [9,14-16]. However, deterioration of coagulation parameters and major bleeding during induction therapy are of critical importance and significantly affect initial mortality. Yanada et al. [16] reported that aggressive transfusion on the day of bleeding achieved the targeted levels of platelet counts (30 × 10^9/L) and fibrinogen (150 mg/dL) in only 71% and 40% of APL patients, respectively. The authors suggested that a more intensive transfusion policy could be beneficial for patients at high risk of hemorrhage, and showed that patients who did not experience hemorrhagic complications had an excellent long-term outcome. Our data showed no correlation between initial bleeding and OS (p = 0.102). In addition, none of the individual coagulation parameters making up the diagnostic criteria of overt DIC proposed by the ISTH and KSTH, including fibrinogen, significantly affected OS. Additional prospective studies with larger numbers of patients are warranted to confirm whether fulfilling the diagnostic criteria of overt DIC affects OS in patients with APL.

In total, 13% of analyzed patients died due to fatal bleeding within the first 14 days of remission induction treatment. In APL treatment, the major cause of treat-ment failure is death during induction therapy. This has ranged from 5% to 10% in recent multicenter trials and most deaths have been the result of hemorrhage, infection, and differentiation syndrome [17]. Our data showed that the mortality rate during induction therapy was 23.9%. This is considerably higher than the results from trials conducted in Europe and the United States, and may be due to a lack of intensive supportive care. Transfusions, antibiotics, and/or antifungal agents are important for supportive care in acute leukemia. From 2005 onwards, the supportive care for APL patients was intensified at our institution with aggressive transfusion and antifungal strategies. As a result, the OS markedly improved after 2005 (Fig. 3).

This study had some limitations, including its retrospective nature and inclusion of patients diagnosed many years ago (from 1995 onwards). In addition, the study population was small because APL has a relatively low incidence and lower prevalence than other types of AML, and the study population originated from a single medical center. Nevertheless, our results represent novel data on the applicability of overt DIC criteria proposed by the ISTH and KSTH for the diagnosis of bleeding tendency in APL, and provide an evaluation of the impact of each parameter on initial bleeding and OS.

In conclusion, the initial fibrinogen level was the only contributing factor among the diagnostic criteria of overt DIC for bleeding presentation in APL patients. Not all of the diagnostic criteria may contribute to manifestation of initial bleeding in APL patients.

KEY MESSAGE

1. Initial fibrinogen level in newly diagnosed patients with acute promyelocytic leukemia (APL) was the only contributing factor among the diagnostic criteria of overt disseminated intravascular coagulation for bleeding presentation.
2. Maintaining a sufficient fibrinogen levels is as important as maintaining platelet levels for preventing hemorrhagic complication in patients with newly diagnosed APL.

Conflict of interest

No potential conflict of interest relevant to this article

was reported.

Acknowledgments

REFERENCES

1. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009;114:937-951.

2. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood 2008;111:2505-2515.

3. Ades 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;111:1078-1084.

4. Tallman MS, Altman JK. How I treat acute promyelocytic leukemia. Blood 2009;114:5126-5135.

5. Choudhry A, DeLoughery TG. Bleeding and thrombosis in acute promyelocytic leukemia. Am J Hematol 2012;87:596-603.

6. Tallman MS, Manji GA. Don't just stand there, do something: strategies for the prevention of early death in acute promyelocytic leukemia: a commentary. Blood Cells Mol Dis 2011;46:173-174.

7. Jacomo RH, Melo RA, Souto FR, et al. Clinical features and outcomes of 134 Brazilians with acute promyelocytic leukemia who received ATRA and anthracyclines. Haematologica 2007;92:1431-1432.

8. Cornell RF, Palmer J. Adult acute leukemia. Dis Mon 2012;58:219-238.

9. Breen KA, Grimwade D, Hunt BJ. The pathogenesis and management of the coagulopathy of acute promyelocytic leukaemia. Br J Haematol 2012;156:24-36.

10. Lee JH, Song JW, Song KS. Diagnosis of overt disseminated intravascular coagulation: a comparative study using criteria from the International Society versus the Korean Society on Thrombosis and Hemostasis. Yonsei Med J 2007;48:595-600.

11. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation: British Committee for Standards in Haematology. Br J Haematol 2009;145:24-33.

12. Rodeghiero F, Avvisati G, Castaman G, Barbui T, Mandelli F. Early deaths and anti-hemorrhagic treatments in acute promyelocytic leukemia: a GIMEMA retrospective study in 268 consecutive patients. Blood 1990;75:2112-2117.

13. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M; Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost 2001;86:1327-1330.

14. de la Serna J, Montesinos P, Vellenga E, et al. Causes and prognostic factors of remission induction failure in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and idarubicin. Blood 2008;111:3395-3402.

15. Cordonnier C, Vernant JP, Brun B, et al. Acute promyelocytic leukemia in 57 previously untreated patients. Cancer 1985;55:18-25.

16. Yanada M, Matsushita T, Asou N, et al. Severe hemorrhagic complications during remission induction therapy for acute promyelocytic leukemia: incidence, risk factors, and influence on outcome. Eur J Haematol 2007;78:213-219.

17. Rego EM, Kim HT, Ruiz-Arguelles GJ, et al. Improving acute promyelocytic leukemia (APL) outcome in developing countries through networking, results of the International Consortium on APL. Blood 2013;121:1935-1943.