A scoring system to predict life-threatening thrombo-ischemic events in patients with acute promyelocytic leukemia: the PETHEMA/PALG study

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Abstract

Acute promyelocytic leukemia (APL) is a highly curable leukemia characterized by life-threatening coagulopathy leading to hemorrhagic and thrombo-ischemic events. We analyzed the incidence, outcomes and risk factors of thrombo-ischemic events in a large series of 1,210 patients with newly diagnosed APL reported to the PETHEMA registry. Therapy consisted of ATRA and chemotherapy (AIDA-based). Median age of patients was 46 years (range 2-90 years). Fifty-eight patients (5%) did not start the AIDA regimen either because they were unfit for chemotherapy, or because of early death before initiating ATRA. A total of 195 (16%) patients developed thrombo-ischemic events, the most frequent being superficial-vein and/or central catheter-related (6.9%) followed by central nervous system (2.2%), deep-vein thrombosis (2.1%), pulmonary embolism (2.1%), acute myocardial infarction (1.6%), or other locations (1.2%). Thrombo-ischemic events mostly occurred at diagnosis and during induction (4.0% and 9.3%, respectively). Patients developing life-threatening thrombo-ischemic events (i.e., excluding superficial and/or catheter-related) at diagnosis / induction had a 31% early death rate. Prolonged activated partial thromboplastin time (aPTT), age >40 years, ECOG performance status >1, platelets >25x10^9/L, and absence of bleeding at presentation were independent risk factors for life-threatening thrombo-ischemic events. Using these variables (1 point each), we developed and validated the Thromb-On risk score, identifying a high-risk group (3-5 points). The Thromb-On risk score was validated in a cohort of 585 patients treated since 2017 with arsenic trioxide plus all-trans retinoic acid (ATRA) (<10x10^9 leukocytes) or according to the AIDA protocol (≥10x10^9 leukocytes). This study could help to improve prevention and management of life-threatening thrombo-ischemic events through risk-adapted guidance, potentially leading to a decrease in early mortality in APL.

Introduction

Acute promyelocytic leukemia (APL) is a highly curable leukemia characterized by the t(15;17) translocation. However, there is still a 10-15% risk of pre-treatment and induction mortality, mostly due to hemorrhages and a frequent association with life-threatening coagulopathy.[1-4] Coagulopathy in APL is not only associated to hemorrhage but to a procoagulant state that could lead to thrombotic and ischemic events, which could also be a fatal complication. The real incidence of thrombosis in APL ranges between 0.5 to 20.6%,[5-13] with prospective studies reporting the higher incidence. Different risk factors for thrombosis development in APL have been proposed, such as differentiation syndrome (DS),[7,12] higher white blood cell (WBC) count,[11-14] bcr-3 type *PML/RARA*,[9,13] *FLT3-ITD* mutation,[9,13] CD2 and CD15[13] expression, tranexamic acid prophylaxis, low fibrinogen and M3 variant subtype.[12] Establishing the incidence and risk factors for thrombo-ischemic events could help to design supportive care guidelines aimed at preventing this life-threatening complication.

The aim of this study is to prospectively analyze the incidence, outcome and risk factors of thrombo-ischemic events in a large series of patients diagnosed with APL and treated with PETHEMA LPA2005 and LPA2012 protocols based on all-trans retinoic acid (ATRA) and chemotherapy. In addition, we aim to analyze the incidence of thrombo-ischemic complications in a large series of patients treated with modern APL regimens (LPA2017 protocol), which included ATO+ATRA for low- and intermediate-risk patients, and the AIDA protocol-based regimen for high-risk patients. This study could help to improve prevention and management of these events through risk-adapted guidance (i.e., based on a validated scoring system) for the management of thrombo-ischemic complications, potentially leading to a decrease in early mortality in APL.

Methods

Study design and population

The study comprises adult and pediatric newly diagnosed APL patients that were enrolled in the PETHEMA APL and acute myeloid leukemia registry (clinicaltrials.gov NCT02607059) between June 2005 and April 2017. To validate the scoring system, we used an external cohort of patients treated with modern protocols since May 2017 with arsenic trioxide (ATO) plus ATRA (if leukocytes <10x10^9 at APL diagnosis) or according to the AIDA protocol (≥10x10^9 leukocytes). All patients with confirmed t(15;17) and/or *PML/RARA* rearrangements and intention to treat were included, regardless of the performance status, or whether they were "*de novo*" or secondary. According to the Declaration of Helsinki, informed consent was obtained from all patients, and the registry and treatments protocols were approved by institutional research ethics committees (2024-0499-1).

Acute promyelocytic leukemia therapy

Therapy for APL was given according to the PETHEMA LPA2005[15] and the LPA2012 trials[16] (ethics approval code 2012/00050/EO). The LPA2017 protocol included ATO+ATRA according to the Lo Coco schedule for low-intermediate risk APL, and AIDA-based for high-risk patients. (See *Online Supplementary Appendix*).

Data collection

The following variables were prospectively collected, among

others, in a specific form: sex, age, performance status using Eastern Cooperative Oncology Group (ECOG) scale, *de novo versus* secondary, baseline fever, body mass index (BMI), body surface, liver and spleen enlargement, thrombosis at diagnosis (yes/no/location); hemorrhage at diagnosis (yes/no/location); fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrin degradation products or D-dimers, albumin, serum lactate dehydrogenase (LDH), creatinine, blood urea nitrogen (BUN), uric acid, alkaline phosphatases, transaminases, cholesterol, triglycerides. total bilirubin; WBC and platelets, peripheral blood blast count and bone marrow blast percentage; bone marrow aspirate cellularity, peroxidase reactivity; local diagnostic laboratory parameters CD13, CD56, CD15, CD34, CD7, CD14, CD117, CD2, CD9, CD19, and CD33 surface antigen markers; *PML/RARA* bcr3 isoform, *FLT3-ITD* mutation, M3 variant, and karyotype. Induction outcome, cause of death, and the occurrence and severity of DS were captured. Data concerning thrombo-ischemic events during induction and consolidation cycles were also collected (yes/no/location/outcome).

Definitions and study endpoints

The primary endpoint was the overall incidence of thrombo-ischemic events during diagnosis, induction, and consolidation phases, including superficial vein and catheter-related thromboses (i.e., non-life-threatening events) and other thrombo-ischemic complications (i.e., life-threatening events). Thrombo-ischemic events were diagnosed by clinical signs or symptoms, based on routine practice. Thrombosis in active APL was considered when patients developed a thrombosis at diagnosis and/or during induction before achieving a complete remission (CR). For risk factors and predictive model analyses, superficial vein and catheter-related thrombosis were not considered events as the presence of vein access was taken to be the main risk factor itself.

Coagulopathy was defined as a prolonged PT and/or aPTT in addition to hypofibrinogenemia and/or increased levels of fibrin degradation products or D-dimers, as well as hypofibrinogenemia with increased levels of fibrin degradation products or D-dimers.

Differentiation syndrome diagnosis was made according to previously published criteria[17] after ruling out other causes. Severe DS was considered when 4 or more of these symptoms were present: fever, dyspnea, pleural or pericardial effusion, pulmonary infiltrates, renal failure, hypotension, weight increase (≥5kg), and other diagnoses were ruled out. Risk of relapse was assessed according to the Sanz predictive model, as reported previously.[18]

The primary endpoint of the study was to assess the overall incidence of thrombosis and its incidence at different timepoints (diagnosis, induction, and consolidation). Secondary endpoints were to analyze risk factors for thrombosis development, overall survival (OS), and early mortality (i.e., mortality before starting therapy and during induction).

Statistical analysis

See *Online Supplementary Appendix*.

Results

Patients' characteristics

A total of 1,210 consecutive patients with a full data set on thrombo-ischemic events were registered in the PETHEMA protocols LPA 2005 (N=941, 77.8%) and LPA 2012 (N=269, 22.2%). Fifty-eight patients (5%) did not start the AIDA regimen either because they were considered unfit for chemotherapy due to underlying comorbidity/ECOG performance status 4 or because of early death before initiating ATRA. Median age of patients was 46 years (range 2-90), secondary APL was diagnosed in 11% (N=133), and ECOG was 0-1 in 76% (N=829) of patients. The Sanz risk score distribution was as follows: low 22%(N=270), intermediate 49% (N=593), and high 29% (N=345). Among all patients, 166 (13.7%) patients died early before starting therapy or during induction.

Incidence, site, and timing of thrombo-ischemic events

A total of 195 (16%) patients developed thrombo-ischemic events; 171 (14%) had one episode and 24 patients (2%) developed more than one episode. Overall, the most frequent thrombotic event was superficial vein and/or central catheter-related thrombosis (SCVT), affecting 84 (6.9%) patients, followed by central nervous system ischemic stroke (CNS) in 27 (2.2%), deep-vein thrombosis (DVT) in 26 patients (2.1%), pulmonary embolism (PE) in 25 (2.1%), acute myocardial infarction (AMI) in 19 (1.6%), and other locations such as Budd-Chiari syndrome or corticorenal or splenic ischemia in 14 (1.2%) (Table 1).

Incidence of thrombo-ischemic events was higher during active APL phases, affecting 162 out of 1,210 patients (13.4%), 49 (4.0%) at diagnosis before starting ATRA, and 113 (9.3%) during induction. During the consolidation phase, 33 (3.2%) patients out of 1,044 developed thrombo-ischemic events. The site and type of thrombo-ischemic event differed between the diagnosis, induction, and consolidation phase (Table 1 and Figure 1). At diagnosis, the type of thrombosis was distributed as follows: CNS 13 (24%), DVT 11 (22%), PE 10 (20%), AMI 9 (18%), and other sites 6 (14%). During induction, the type of thrombosis was distributed as follows: SCVT 58 (51%), CNS 14 (12%), DVT 13 (12%), PE 13 (12%), AMI 9 (8%), and other sites 6 (5%). In the course of consolidation phases, the type of thrombosis was distributed as follows: SCVT 26 (79%), DVT 2 (6%), PE 2 (6%), AMI 1 (3%), and other sites 2 (6%).

Risk factors for life-threatening thrombosis in active acute promyelocytic leukemia

There were 104 (9%) of episodes of life-threatening thrombo-ischemic events during active APL (i.e., at diagnosis and/or induction and excluding SCVT). Table 2 shows univariate

analyses comparing patients in active APL phase with and without life-threatening thrombosis. The following factors were related to a higher incidence of life-threatening thrombosis at diagnosis and/or during induction phase: older age (*P*=0.004), higher BMI (*P*=0.02), higher weight (*P*=0.007), Sanz low-risk (*P*=0.01), higher ECOG performance status (*P*<0.001), higher platelet counts (*P*<0.001), hypoalbuminemia (*P*=0.001), prolonged aPTT (P<0.001), triglycerides ≥220 mg/dL (*P*=0.03), higher creatinine levels (*P*=0.02), >20% blasts CD56 expression (*P*=0.005), and absence of hemorrhagic signs or symptoms at diagnosis (*P*<0.001). A trend was observed with cholesterol ≥200 mg/dL (*P*=0.07), and higher urea levels (*P*=0.06). No significant relation was observed with CD2 or other surface markers, as well as FLT3 mutations or any other characteristic.

The multivariate analysis showed the following independent risk factors: age over 40 years (*P*=0.03), platelet count >25x10^9/L (*P*=0.03), absence of hemorrhage at diagnosis

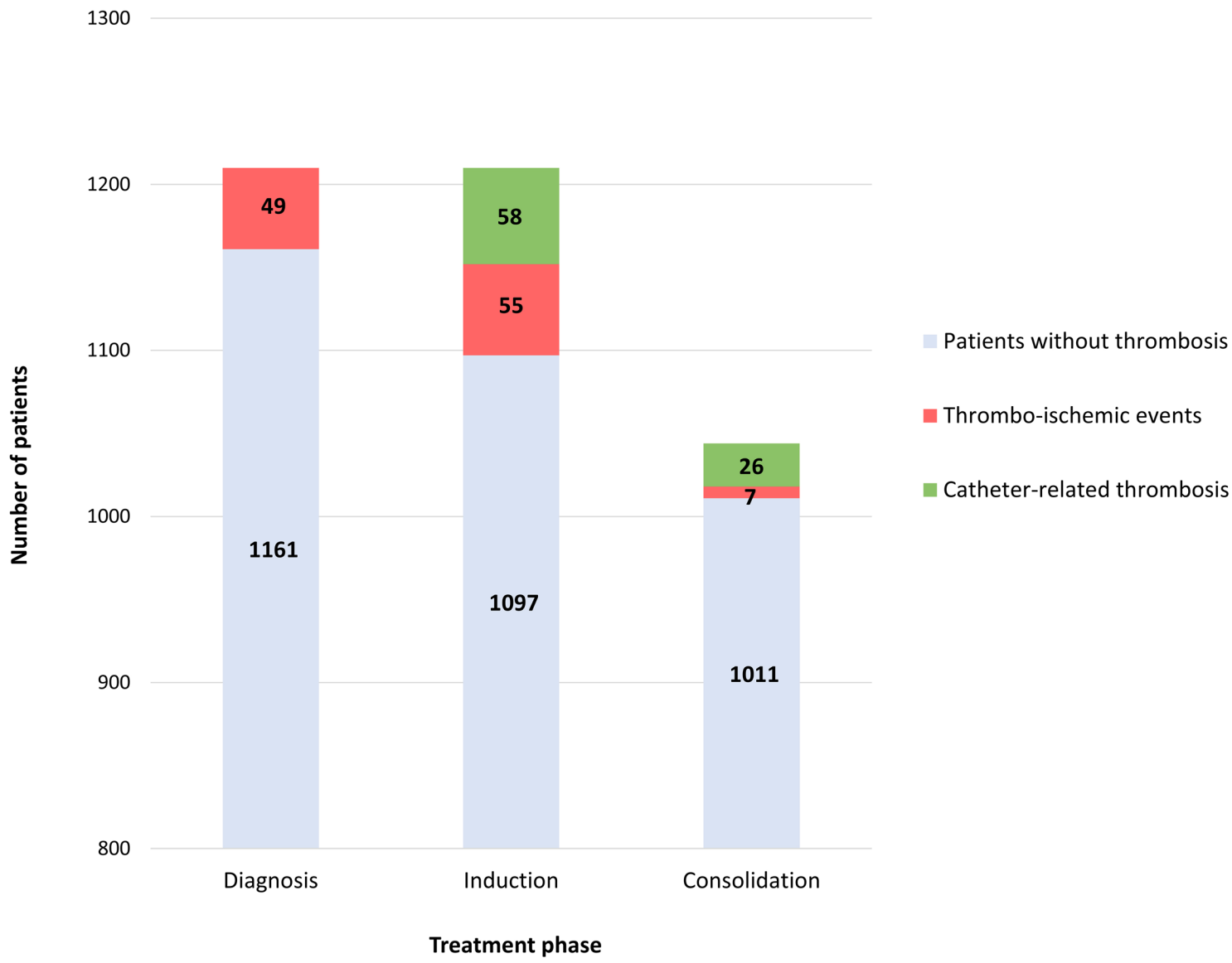


Figure 1. Incidence of thrombosis according to treatment phase and type of thrombosis. Treatment phase: diagnosis, induction, consolidation. Type of thrombosis: catheter-related/surface vein thrombosis *versus* life-threatening thrombo-ischemic events. Data refer to patients treated with AIDA-based regimen.

Table 1. Location of all thrombo-ischemic events according to treatment phase.

Location	Overall N (%)	At diagnosis N (%)	Induction N (%)	Consolidation N (%)
Overall thrombosis	195 (100)	49 (100)	113 (100)	33 (100)
Central nervous system	27 (14)	13 (27)	14 (12)	0 (0)
Myocardial infarction	19 (10)	9 (18)	9 (8)	1 (3)
Pulmonary embolism	25 (13)	10 (20)	13 (12)	2 (6)
Deep vein thrombosis	26 (13)	11 (22)	13 (12)	2 (6)
Surface vein or catheter-related	84 (43)	0 (0)	58 (51)	26 (79)
Other sites	14 (7)	6 (12)	6 (5)	2 (6)

Table 2. Patients and acute promyelocytic leukemia characteristics in patients with or without life-threatening thrombosis in active acute promyelocytic leukemia phase at diagnosis or during induction.

Characteristic	Patients without life-threatening thrombosis		Patients with life-threatening thrombosis		*P*
	Mean (range)	N (%)	Mean (range)	N (%)	
Overall; N=1,210	-	1,106 (91)	-	104 (9)	
Sex	-	-	-	-	-
Female	-	563 (51)	-	45 (43)	0.17
Male	-	543 (49)	-	59 (57)	-
Type	-	-	-	-	-
de novo	-	986 (89)	-	91 (88)	0.73
Secondary	-	120 (11)	-	13 (13)	-
Age, years; N=1,206	45 (2-90)	-	51 (9-82)	-	0.002
≤18	-	71 (6)	-	3 (3)	0.006
19-40	-	380 (34)	-	21 (20)	-
41-60	-	412 (37)	-	51 (50)	-
61-70	-	155 (14)	-	15 (15)	-
>70	-	85 (8)	-	13 (13)	-
BMI; N=1,066	27 (13-58)	-	29 (19-48)	-	0.02
Weight, kg; N=1,077	75.4 (12.3-166)	-	81.9 (42-160)	-	0.007
<70	-	611 (62)	-	43 (50)	0.045
≥70	-	380 (38)	-	43 (50)	-
Relapse risk; N=1,208	-	-	-	-	-
Low	-	236 (21)	-	34 (33)	0.004
Intermediate	-	557 (51)	-	36 (34)	-
High	-	311 (28)	-	34 (33)	-
ECOG performance status; N=1,089	-	-	-	-	-
0-1	-	774 (78)	-	55 (60)	<0.001
2	-	116 (12)	-	23 (25)	-
3	-	63 (6)	-	8 (9)	-
4	-	44 (4)	-	6 (7)	-
PETHEMA trial	-	-	-	-	-
LPA 2005	-	857 (77)	-	84 (81)	0.52
LPA 2012	-	249 (23)	-	20 (19)	-
Leukocytes, x10^9/L; N=1,209	2.90 (0.1-217)	-	2.95 (0.5-198.6)	-	0.39
≤20	-	908 (82)	-	81 (78)	0.28
>20	-	197 (18)	-	23 (22)	-
Platelet count, x10^9/L; N=1,207	35 (1-290)	-	51 (1-208)	-	<0.001
≤20	-	478 (43)	-	30 (29)	0.006
>20	-	625 (57)	-	74 (71)	-
Hemoglobin, g/dL; N=1,205	9.3 (1.9-17.7)	-	9.6 (4.1-14.5)		0.30
Albumin, g/dL; N=978	4.1 (2-6)	-	4.1 (1.8-5.1)	-	0.002
≤3.5	178 (20)	-	30 (36)	-	0.001
>3.5	716 (80)	-	54 (64)	-	-
aPTT; N=1,095	-	-	-	70 (76)	-
Normal	-	877 (87)	-		<0.001
Prolonged	-	126 (13)	-	22 (24)	-
Triglycerides, mg/dL; N=706	182 (117-218)	-	225 (56-1,309)	-	0.2
<220	-	476 (75)	-	45 (63)	0.03
≥220	-	158 (25)	-	27 (38)	-
Cholesterol, mg/dL; N=779	179 (150-205)	-	184 (155-215)	-	0.2
<200	-	498 (71)	-	45 (60)	0.07
≥200	-	206 (29)	-	30 (40)	-
Creatinine, mg/dL; N=1,145	0.86 (0.25-6.17)	-	1.02 (0.3-6.17)	-	0.02
<1.3	-	1,002 (96)	-	87 (90)	0.02
≥1.3	-	46 (4)	-	10 (10)	-

Continued on following page.

Characteristic	Patients without life-threatening thrombosis		Patients with life-threatening thrombosis		*P*
	Mean (range)	N (%)	Mean (range)	N (%)	
Urea, mg/dL; N=860	-	-	-	9 (13)	-
<40	-	187 (24)	-	60 (87)	0.06
≥40	-	604 (76)	-		-
CD56+ >20% over blast population; N=771	-	-	-	-	-
<20%	-	631 (89)	-	46 (77)	0.005
≥20%	-	76 (11)	-	15 (23)	-
Bleeding at diagnosis; N=1,169	-		-	-	-
Present	-	808 (76)	-	44 (44)	<0.001
Absent	-	260 (24)	-	57 (56)	-

aPTT: activated partial thromboplastin time; BMI: body mass index; ECOG: Eastern Cooperative Oncology Group; N: number.

(*P*=0.005), prolonged aPTT (*P*=0.02), and ECOG performance status ≥2 (*P*=0.03), remained as independent prognostic factors (Table 3).

Testing and validation of the Thromb-On score predictive model

Based on the odds ratio for each independent risk factor, we assigned 1 point to each factor to build the Thromb-On score system to predict life-threatening thrombosis. Patients were grouped into the following categories: low-risk (0 points), intermediate-risk (1-2 points), and high-risk (3–5 points). The study population where all risk factors were available (N=1,010, 83.5%) was divided into two cohorts using a 1:1 random function. The evaluable population was distributed in low- (13.3%), intermediate- (63.4%) and high- (23.3%) risk. The training cohort, that was used to test the score system, showed 9% of life-threatening thrombosis, and the validation cohort (used for internal validation) showed 8.8%. The risk of life-threatening thrombosis for low-, intermediate-, and high-risk groups was 1.4%, 4.9%, and 23.2%, respectively, in the training cohort, and 3%, 5.8%, and 16.5%, respectively, in the validation cohort (Table 4). The area under the ROC curve was 0.69 when applying the low-intermediate-high risk categories, and 0.70 when applying the sum score categories (0-5 points) in the whole cohort (Figure 2A and B, respectively).

Outcomes in patients with life-threatening thrombosis in active acute promyelocytic leukemia

Early death (during induction and/or in the first month after diagnosis) was more frequent in patients developing life-threatening thrombo-ischemic complications as compared with those who did not (N=32/104 [31%] *vs*. N=134/1,106 [12%], respectively; *P*<0.001). In this group, death was mostly caused by thrombosis (N=19, 18%), followed by hemorrhagic transformation of the thrombosis (N=4, 4%), and other hemorrhages (N=2, 2%) (Figure 3A). Bleeding (N=76, 7%) was the main cause of early death among patients without life-threatening thrombosis in active APL phase (Figure 3B). Patients who developed life-threatening thrombosis had longer duration of hospitalizations during induction (>30 days stay, 9% *vs*. 2%; *P*=0.01). No difference was observed in the incidence of severe DS (85% *vs*. 88%; *P*=0.43) or use of intravenous antibiotic therapy (Table 5).

External validation of the Thromb-On score in patients treated with the LPA2017 protocol

A total of 585 consecutive patients with a full data set on thrombo-ischemic events were registered in the PETHEMA LPA2017 protocol. Twenty-seven patients (4.6%) did not start the regimen either because they were considered unfit for chemotherapy due to underlying comorbidity/ECOG performance status 4 or because they died early before initiating ATRA. Median age was 48 years (range 1-88), secondary APL was diagnosed in 9% (N=51), and ECOG performance status was 0-1 in 87% (N=508) of patients. Median leukocyte count at diagnosis was $2.0x10^9$/L (range 0.14-418.7), with 18% of patients presenting with WBC counts >$20x10^9$/L. Median platelet count was $30x10^9$/L (range 2-269), with 69% presenting with counts >$20x10^9$/L. According to the Sanz risk score, 75% (N=438) of patients were classified as low-intermediate risk, and 25% (N=147) as high-risk. Overall, 76% (N=425) received an ATO+ATRA schedule, whereas 24% (N=135) were treated with an AIDA regimen. Other baseline characteristics are described in *Online Supplementary Table S1*. Among all patients, 82 (14%) died early before starting therapy or during induction. A total of 81 (13.8%) patients developed thrombo-ischemic events, the most frequent being SCVT (6.2%), followed by CNS (3.1%), PE (2.7%), and DVT (1.2%) (*Online Supplementary Table S2*). Incidence of thrombo-ischemic events was higher during active APL phases, affecting 75 out of 585 patients (12.8%), 28 (4.7%) at diagnosis before starting ATRA, and 47 (8%) during induction. Among the subgroup of patients who received the ATO-ATRA regimen (N=425), 49 (11.5%) experienced a thrombotic event at some point during treatment, with 15 events (31%) occurring at diagnosis, 29 (59%) during induction, and 5 (10%) during consolidation. At

diagnosis in this subgroup, the most frequent thrombotic events were PE (47%) and CNS events (33%), whereas during induction and consolidation, SCVT predominated (69% and 80%, respectively). A detailed description of the site and frequency of thrombo-ischemic events in the ATO-ATRA cohort is provided in *Online Supplementary Table S3*.

There were 43 (7.4%) episodes of life-threatening thrombo-ischemic events during active APL (i.e., at diagnosis and/or induction and excluding SCVT). The external validation cohort was distributed in low- (10.9%), intermediate- (66.5%) and high- (22.6%) risk, according to the Thromb-On score. The risk of life-threatening thrombosis for low-, intermediate-, and high-risk groups was 1.6%, 7.2%, and 10.6%, respectively (*Online Supplementary Table S3*). The area under the ROC curve was 0.69 when applying the sum score categories (0-5 points) and 0.58 when applying the low-intermediate-high risk categories, in the external validation cohort (*Online Supplementary Figure S1A* and *B*, respectively).

Early death (during induction and/or in the first month after diagnosis) was more frequent in patients developing life-threatening thrombo-ischemic complications as compared with those who did not (N=13/43 [30.2%] *vs*. N=70/542 [12.9%], respectively; *P*=0.009).

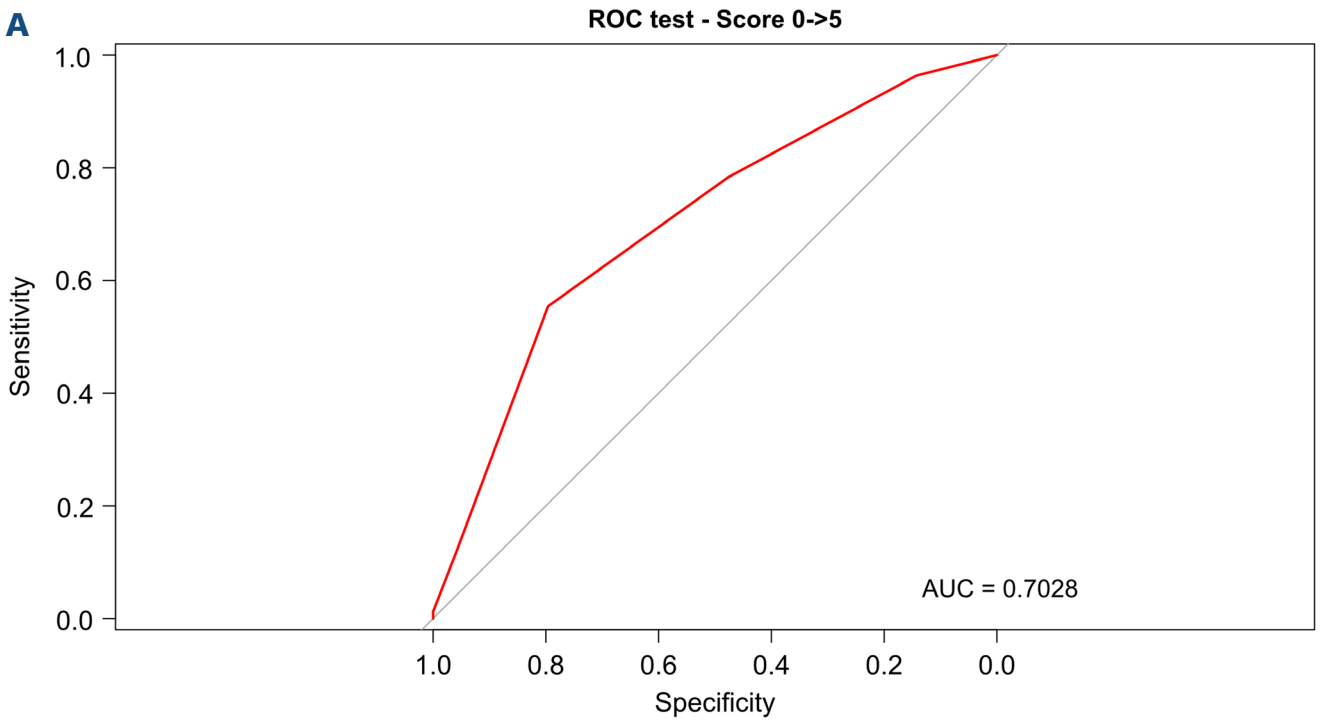
Discussion

This study shows that thrombo-ischemic events are frequent at diagnosis, during induction and consolidation phase in APL patients treated with ATRA-based schedules. Thrombotic complications were mostly life-threatening when occurring before starting ATRA or during induction phase (i.e., with active APL), and their incidence and severity

Table 3. Multivariate analyses of risk factors for development of life-threatening thrombosis in acute promyelocytic leukemia.

Characteristic	Total number of patients N (%)	Life-threatening thrombo-ischemic events N (%)	Odds ratio (95%CI)	*P*
Overall	1,210	104	-	-
Age, years ≤40 >40	 475 (39) 731 (61)	 24 (23) 79 (77)	 - 2.5 (1.1-5.6)	0.03
Platelet count, x10^9/L ≤ 25 >25	 732 (61) 475 (39)	 47 (45) 57 (55)	 - 2.3 (1.1-4.7)	0.03
ECOG performance status 0-1 ≥ 2	 829 (76) 260 (24)	 55 (60) 37 (40)	 - 2.8 (1.4-5.7)	0.005
aPTT Normal Prolonged	 947 (86) 148 (14)	 70 (76) 22 (24)	 - 2.6 (1.2-5.9)	0.02
Bleeding at diagnosis Present Absent	 865 (74) 304 (26)	 57 (56) 44 (44)	 - 2.4 (1.2-4.9)	0.03
Relapse risk Low Intermediate-High	 270 (22) 938 (78)	 34 (33) 70 (67)	 - 1.6 (0.8-3.4)	0.20
Creatinine, mg/dL <1.3 ≥1.3	 1,089 (95) 56 (5)	 87 (90) 10 (10)	 - 1.1 (0.4-3.3)	0.81
Albumin, g/dL ≤3.5 >3.5	 208 (21) 770 (79)	 30 (36) 54 (64)	 - 1.7 (0.9-3)	0.07
Weight, kg <70 ≥70	 654 (61) 423 (39)	 43 (50) 43 (50)	 - 1.2 (0.7-2)	0.58
BMI	27 (13-58)	29 (19-48)	1 (0.9-1)	0.26

aPTT: activated partial thromboplastin time; BMI: body mass index; CI: confidence interval; ECOG: Eastern Cooperative Oncology Group; N: number.



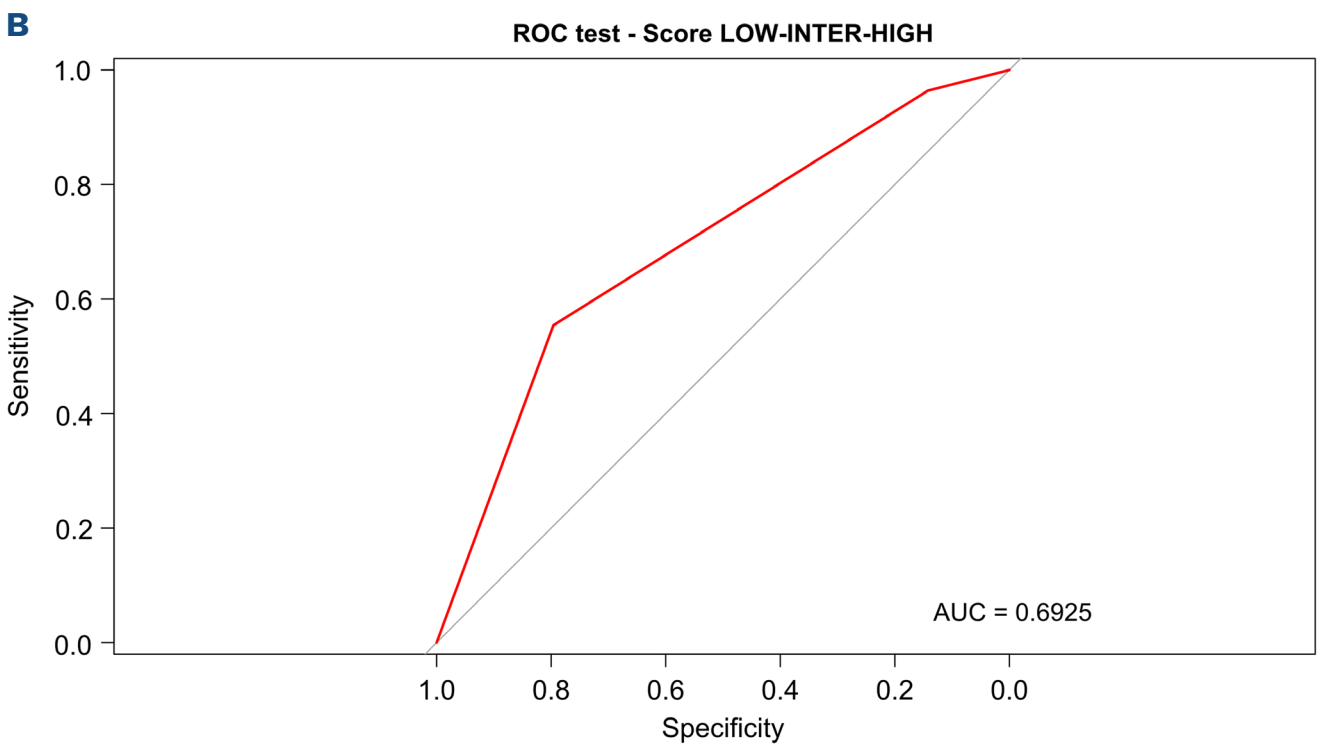


Figure 2. Receiver operating characteristic curve according to the Thromb-On score. (A) Using the sum score 0-5, AUC 0.70. (B) Using the 3 risk categories (low 0 points, intermediate 1-2 points, and high 3-5 points, AUC 0.69). Data refer to patients treated with AIDA-based regimen. AUC: area under the curve; ROC: receiver operating characteristic.

Table 4. Distribution of Thromb-On score risk sum and categories and performance in training and validation cohorts.

Characteristic	All evaluable patients N (%)	Training cohort N=505		Validation cohort N=505	
		No thrombosis N	Thrombosis N (%)	No thrombosis N	Thrombosis N (%)
Overall	1,010 (100)	463	42 (9)	464	41 (8.8)
Sum score					
0 points	135 (13.3)	69	1 (1.4)	63	2 (3.1)
1 point	325 (32.2)	157	6 (3.7)	153	9 (5.6)
2 points	315 (31.2)	154	10 (6.1)	142	9 (6.0)
3 points	186 (18.4)	66	19 (22.3)	84	17 (16.8)
4 points	48 (4.8)	17	6 (26.1)	22	3 (12.0)
5 points	1 (0.1)	0	0 (NA)	0	1 (100.0)
Risk group					
Low (0 points)	135 (13.3)	69	1 (1.4)	63	2 (3.0)
Intermediate (1-2 points)	640 (63.4)	311	16 (4.9)	295	18 (5.8)
High (3-5 points)	235 (23.3)	83	25 (23.2)	106	21 (16.5)

N: number; NA: not applicable.

decreased during consolidation phase (where they were mostly catheter-related). The development of life-threatening thrombosis at diagnosis or during induction leads to prolonged hospitalization and a higher early death rate. We identified 5 independent risk factors that have helped to build and internally validate a simple scoring system: the Thromb-On score. The Thomb-On score could be useful for prevention and management of life-threatening thrombo-ischemic events in APL.

Despite the improvement in survival in APL patients since the introduction of ATRA-based regimens, early death remains the unsolved issue for APL patients and the more challenging cause of treatment failure. The reported early death rate in real-world practice ranges between 10% and 18%,[15,19,20] with bleeding as the most frequent cause of death. So far, the incidence and morbidity-mortality of thrombo-ischemic complications has been analyzed in relatively small and/or retrospective studies, reporting a wide range of thrombosis from 0.5 to 20.6%.[5-13] We identified only one prospective study that included 31 APL patients and showed 9.6% thrombosis.[21] On the other hand, two recent retrospective studies including 248 and 364 patients treated with ATO+ATRA, showed an incidence of thrombosis of 5% and 0.5% respectively.[8,22] To date, the largest study included 759 patients, data were retrospectively collected in the context of PETHEMA LPA96 and LPA99 protocols, and

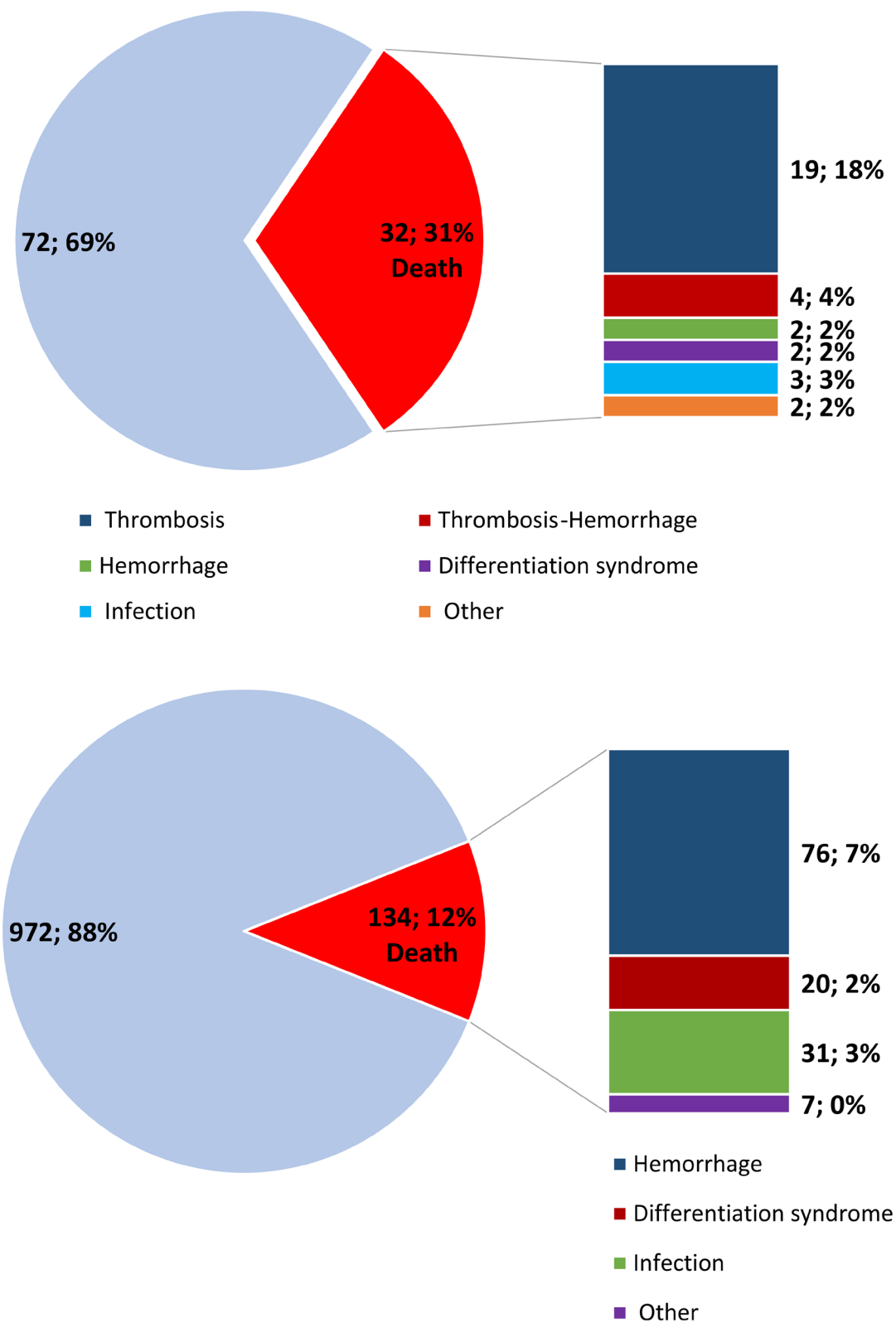


Figure 3. Early death rate and causes of death in acute promyelocytic leukemia patients. (A) Patients who developed life-threatening thrombosis at diagnosis or during induction. (B) Patients who did not develop life-threatening thrombosis at diagnosis or during induction. Data refer to patients treated with AIDA-based regimen.

the reported incidence was 5.1%.[12] The present study has collected data prospectively and it includes a large series of patients treated homogenously in the LPA2005 and the LPA2012 PETHEMA trials. In addition, we have investigated the incidence of thrombo-ischemic complications in a large series of 585 patients treated with modern APL protocols (i.e., LPA2017, incorporating ATO+ATRA for patients with <10x10^9 WBC).

Catheter-related thrombosis is a well-known risk factor for thrombosis development.[23,24] In our study, 43% of thrombotic events were associated with a venous line. Interestingly, a study performed in 25 patients reported 32% of catheter-related thrombosis in APL.[25] In the case of catheter-related thrombosis, the risk of thrombosis was attributed to the catheter itself and, therefore, these patients were excluded from the analysis of risk factors. Additionally, patients that developed thrombosis during consolidation were not considered as events for the risk factor analyses, assuming that, in this phase, thrombosis was not related to APL itself, but rather to general population risk factors (e.g., prolonged hospitalization or catheter-related). Another well-known risk factor for thrombosis is thrombophilia,[26] and this has also been associated with thrombosis in one study that included 34 patients;[6] but in our real-world series, we have not collected thrombophilia studies as they are not routinely performed in APL.

Here, we focused on the analysis of risk factors for developing life-threatening (i.e., no superficial vein or catheter-related) thrombosis, which included CNS, DVT, PE, AMI, and other severe complications at diagnosis or during induction, affecting 9% of 1,210 patients. Indeed, we found that these life-threatening thrombo-ischemic events were related to morbidity (prolonged hospitalization) and higher early death rate (up to 31%). Interestingly, in the LPA2017 cohort, we found similar rates of life-threatening thrombo-ischemic events (7.4%), leading also to a higher mortality rate (30.2%) in this group, suggesting that switching to ATO+ATRA therapy, at least for patients with <10x10^9 WBC, did not impact on the incidence and severity of this complication.

The introduction of ATRA into APL therapy has produced a high rate of CR with a rapid resolution of coagulopathy. It has been postulated that the imbalance caused by ATRA between procoagulant and fibrinolytic forces may induce a prothrombotic effect,[7,27] but there seem to be additional risk factors for thrombosis in the APL active phase. In this regard, it is important to highlight that we have found up to 4% of thrombosis at diagnosis, prior to the onset of ATRA, confirming that there are stronger biological factors than the procoagulant state induced by differentiating agents. Another risk factor that has been related to the exacerbation of the procoagulant state is the development of DS, as suggested in a study performed on 31 patients,[7] and in a study analyzing DS in 780 patients in which severe DS was associated with a higher incidence of thrombotic events.[12] However, this PETHEMA study analyzed retrospective data of thrombosis. In this larger and specifically designed study, we could not confirm any association between severe DS and life-threatening thrombosis.

Other factors have been associated with thrombosis, such as leukocytosis ATRA syndrome and some laboratory data specific to this disease: higher platelet count,[9] ISTH DIC score,[9] APL variant M3,[12] PAI 4G/4G,[9] CD2 and CD15.[13] However, most of these factors have not been confirmed by others. The following prognostic factors were identified in at least two studies: leukocytosis, hypofobrinogenemia, PT, aPTT, D-dimers, *FLT3-ITD*, and the bcr3 isoform.[8,9,12-14,22] In

Table 5. Outcomes of acute promyelocytic leukemia patients with and without life-threatening thrombo-ischemic events at diagnosis or during induction.

Characteristic	Patients without thrombosis		Patients with thrombosis in active APL		*P*
	Median (range)	N (%)	Median (range)	N (%)	
Overall; N=1,210	-	1,106 (91)	-	104 (9)	-
Death during induction; N=1,209					
No	-	972 (88)	-	61 (69)	<0.001
Yes	-	134 (12)	-	32 (31)	
Hospitalization during induction, days; N=871					
≤30	-	796 (98)	-	51 (91)	0.01
>30	-	19 (2)	-	5 (9)	
Days under intravenous antibiotics during induction; N=947	19 (1-161)	-	18 (2-59)	-	
≤15	-	426 (49)	-	37 (45)	0.55
>15	-	439 (51)	-	45 (55)	
Differentiation syndrome; N=1,148					
Severe	-	119 (11)	-	14 (15)	0.43
No/moderate	-	993 (88)	-	82 (85)	

APL: acute promyelocytic leukemia; N: number.

the present study, none of those factors were confirmed in the multivariable analysis with the exception of prolonged aPTT, identified in two previous studies.[9,22] Other independent risk factors were age >40 years, ECOG performance status >1, platelets >25x10^9/L, and absence of bleeding at APL presentation. Based on these parameters we could identify a high-risk population for life-threatening thrombosis, and we built and validated a simple scoring system with acceptable AUC of the ROC curve (0.7 in the whole LPA2005 and LPA2012 cohort, and 0.69 in the modern LPA2017 cohort). To our knowledge, no risk scores for thrombosis in APL have been published to date. We identified 23.3% of the APL population with a high risk for development of life-threatening thrombo-ischemic events, for whom we could recommend frequent monitoring, and a high suspicion of thrombosis with compatible signs or symptoms; even anticoagulant prophylactic strategies could be used in the context of well-designed protocols. In addition, platelet and plasma product transfusion policies could be more restrictive in this setting. For low Thromb-On score patients, management should focus on preventing bleeding, and for the intermediate-risk group, we would suggest following the current supportive measures adopted by the European LeukemiaNet (ELN) expert panel.[28]

Limitations in our study are: 1) not all registered patients had a full data-set regarding thrombosis events, and selection bias cannot ruled out; 2) we did not include patients with ≥10x10^9 WBC treated with ATO in front-line, potentially restricting the applicability of our score system as chemotherapy-free regimens become the standard also in this population; and 3) the score system should be validated in additional external cohorts.

In conclusion, in this large study analyzing real-world APL patients, thrombo-ischemic events were frequent, mostly occurring in life-threatening locations, developing at diagnostic and induction phase, and leading to an increased early death rate. The simple Thromb-On score identifies patients with high risk of life-threatening thrombosis, and it could offer guidance for prevention and early management. Further investigation to reduce early death in APL patients is warranted.

Disclosures

No conflicts of interest to disclose.

Contributions

RR-V and PM conceived the study, and analyzed and interpreted the data; PM, RR-V and MS wrote the paper; PM and PL performed the statistical analyses; all of the other authors reviewed the manuscript and contributed to the final draft.

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Data-sharing statement

Data could be shared on reasonable request, by contacting the registry co-ordinator (montesinos_pau@gva.es).

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