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Incidence of thrombosis in adults with acute leukemia: a single center experience in Mexico

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ABSTRACT

Background. Acute leukemias are hematopoietic malignancies that may be accompanied by hemostatic abnormalities. In general, information on the frequency of thrombotic events, their clinical characteristics and survival in adult patients with acute leukemia is still scarce and controversial. **Objectives.** To describe the frequency of thrombotic events, their clinical characteristics and survival of adult patients with acute leukemia at the *Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City*. **Material and methods.** A patient cohort, diagnosed and treated between October 2003 and December 2009, was retrospectively analyzed in terms of thrombotic events, frequencies and survival curves. **Results.** We analyzed 181 patients with a median age of 33 years, 80 were female (44.2%). Fifteen cases with thrombosis (8.3%) were documented and in 53.3% of cases, they were related to the use of a central venous catheter. The median time to development of thrombosis was 92 days; 33.3% of events occurred during the first 30 days after diagnosis. The incidence of thrombosis in patients receiving L-asparaginase was 15%. Of the 15 patients with thrombosis, 27% were alive and without evidence of disease at last follow-up, and 73% had died; disease progression was the most common cause of death (81.8%). None of the thrombotic events had an impact on mortality. Median overall survival (OS) was 349 days. **Conclusions.** The incidence of thrombosis in this adult acute leukemia population is comparable to that reported in the literature. Only a third of cases occurred during the first month after diagnosis; however, 93.3% of patients developed a thrombotic event during the first year after the diagnosis of acute leukemia. All cases were symptomatic and central venous catheter-related thrombosis was the most frequent presentation in this group. Survival curves comparing patients with and without thrombosis were similar. Prospective studies are necessary in order to assess the risk factors fostering thrombosis in adult patients with acute leukemia.

Incidencia de trombosis en pacientes adultos con leucemia aguda: experiencia de un centro de referencia en México

RESUMEN

Antecedentes. *Las leucemias agudas son neoplasias hematopoyéticas que pueden cursar con anormalidades en la hemostasia. En general, la información acerca de la frecuencia de eventos trombóticos, características clínicas e impacto en la supervivencia de pacientes adultos con leucemia aguda es escasa y controversial.* ***Objetivos.*** *Describir la frecuencia de eventos trombóticos, características clínicas y supervivencia de la población adulta con leucemia aguda tratada en el Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), en la Ciudad de México.* ***Material y métodos.*** *Estudio de cohorte retrospectiva, de octubre de 2003 a diciembre de 2009. Se evaluaron las características clínicas, episodios de trombosis y supervivencia global.* ***Resultados.*** *Se estudiaron 181 pacientes con una mediana de edad de 33 años, 44.2% del género femenino. Se documentaron 15 casos de trombosis (8.3%), de los cuales 53.3% estuvo relacionado con el uso de un catéter venoso central. La mediana de tiempo para desarrollar trombosis fue de 92 días, en 33.3% de los casos ocurrió en el primer mes de diagnóstico. La incidencia de trombosis en pacientes tratados con L-asparaginasa fue de 15%. De los 15 pacientes con trombosis, 27% estaba vivo sin evidencia de enfermedad al momento del último seguimiento, y 73% murió; la progresión de la enfermedad fue la causa más frecuente (81.8%). No se reportaron episodios de trombosis recurrente y ninguno de los eventos tuvo repercusiones en la mortalidad. La mediana de supervivencia global (SG) fue de 349 días.* ***Conclusiones.*** *La incidencia de trombosis en nuestra cohorte es comparable con lo publicado en la literatura internacional. Un tercio de los casos presentó trombosis durante el primer mes del diagnóstico; sin embargo, de los casos que presentaron un evento trombótico, 93.3% lo presentó en el primer año posterior al diagnóstico de leucemia aguda. Todos los casos fueron sintomáticos y la trombosis asociada a catéter venoso central fue el evento más frecuente. Las curvas de SG fueron similares en pacientes con y sin trombosis. Se requie-*

ren estudios prospectivos que permitan evaluar los factores de riesgo relacionados al evento trombótico en pacientes con leucemia aguda.

Key words. Acute. Leukemia. Thrombosis. Survival. Adults.

Palabras clave. *Leucemia aguda. Trombosis. Supervivencia. Adultos.*

INTRODUCTION

The association between cancer and thrombosis is well established. The first described hemostatic abnormalities in oncological diseases are hemorrhage, thrombosis and a prolonged bleeding time.[1] Thrombotic events are common complications in cancer patients; some studies have shown a 2 to 7-fold increased risk of thrombosis in this population.[2]

In solid tumors and lymphomas, deep venous thrombosis (DVT) and pulmonary embolism (PE) are the most common cancer-associated thrombotic events[3] and up to 15% of cases may precede the diagnosis of malignancy.[4]

The estimated annual incidence of venous thromboembolism (VTE) in cancer patients is 0.5% compared to 0.1% in the general population.[5,6] In the United States of America, thrombosis is considered the second leading cause of death in cancer patients beginning chemotherapy,[7] however there is scarce information about the impact on mortality in hematologic malignancies.

Acute leukemias are clonal hematopoietic malignancies and patients may present a wide spectrum of hemostatic alterations translating into a high risk of thrombotic and/or hemorrhagic complications.[8,9]

In these patients, the incidence of thrombosis ranges between 2 and 36%.[10-14] At diagnosis, the incidence of venous thrombosis is estimated to be in the range of 1.4 to 9.6% and this incidence increases 1.7 to 12% during induction treatment.[15] Of note, the variation in the incidence of thrombosis depends on several factors, such as those related to the study design (prospective *vs.* retrospective), the administered chemotherapy, the leukemia subtype and concomitant thrombophilia, among others.[16]

Relevant aspects regarding its pathophysiology have been previously addressed by several study groups. Bleeding diathesis due to enhanced fibrinolytic activity through up-regulation of annexin-2 in promyelocytes has been described in acute promyelocytic leukemia (APL) patients, and resolves upon treatment with all-trans-retinoic-acid (ATRA).[17] An increased synthesis of tumor necrosis factor- alpha (TNF-α) and interleukin-1 (IL-1) that subsequently act on the endothelium releasing tissue factor (TF) and plasminogen activator inhibitor (PAI-1),[18] are some of the abnormalities involved in the pathogenesis of thrombosis in cancer. Failure to inactivate factors V and VIII due to a decrease in endothelial thrombomodulin, part of the protein C system, has also been reported as a predisposing factor to thrombosis in cancer.[19] It has been postulated that induction chemotherapy directly damages the endothelium and promotes a procoagulant state in response to the release of endothelial cytokines.[20] Particularly in acute lymphoblastic leukemia (ALL), L-asparaginase reduces the levels of coagulation factors VIII, XI, fibrinogen and vitamin K-dependent factors (II, VII, IX, X) promoting bleeding. L-asparaginase also reduces the levels of anticoagulant proteins (C, S, antithrombin) and plasminogen, thus favoring thrombosis.[21] This effect appears to be enhanced by the concomitant use of steroids and L-asparaginase in children with ALL, an established prothrombotic risk factor.[22]

The use of intravenous devices, specifically central venous catheters (CVC), is a well-documented risk factor in the development of thromboses.[12,23] Other potential variables have also been proposed and include total leukocyte count (WBC > 11 x 10^9/L), platelet count (> 350 x 10^9/L), [24] TF levels, D-dimer, C-reactive protein and soluble P-selectin concentrations.[25]

Although the association between malignancy and thrombosis is well established in white populations,[12] little is known about its frequency, clinical characteristics and outcome in adult acute leukemia patients in Latin American countries.[26] Therefore, the aim of this study is to describe the frequency of thrombotic events, clinical characteristics and survival curves of adults with acute leukemia treated at a tertiary care center in Mexico City.

MATERIAL AND METHODS

Patients and study design

All acute leukemia patients diagnosed at the *Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ)* between October 2003 and December 2009, were included. We conducted a

retrospective analysis of available clinical and laboratory data upon leukemia diagnosis and during the thrombotic event. We included patients with objectively documented thrombosis by any imaging method (CT scan or Doppler ultrasound). Superficial thrombophlebitis episodes were not included in the study. We also recorded all pertinent clinical data, the use of intravenous CVC, surgical events within the previous 30 days, a history of hereditary thrombophilia, smoking habit, physical activity and prolonged hospitalization.

Patients lost before the last follow-up (December 31st, 2009) were excluded from analysis (eleven cases). Data collection underwent institutional Board review and approval. Since the project was deemed to qualify as minimal risk research, the Board granted waivers for informed consent after the demonstration of adequate privacy safeguards.

Definitions

The diagnosis of acute leukemia was established according to the criteria of the World Health Organization (WHO)[27] and immunophenotyping by flow cytometry of bone marrow samples or peripheral blood, available in most cases. Cytogenetic analysis was performed in bone marrow samples, as well as fluorescence in situ hybridization (FISH) for *BCR/ABL* and *PML/RAR*α in ALL and APL patients, respectively.

Tumor lysis syndrome was established when 4 or more of the following were present: hyperkalemia (K $\geq$ 4.5 mEq/L), hyperuricemia (uric acid $\geq$ 5.5 mg/dL), hyperphosphatemia (P $\geq$ 4.3mg/dL), hypocalcemia (Ca $\leq$ 8 mg/dL), increased levels of lactate dehydrogenase, and a rise in creatinine above the normal upper limit. Liver function tests (LFTs) were considered abnormal when transaminases or bilirubin were $\geq$ 2.5 times above the upper normal value. *Prolonged hospitalization* was defined as a period $\geq$ 15 days and *sedentary* as the lack of regular physical activity at least 2 times per week.

We defined *poor prognosis ALL* when one or more of the following were present: hyperleukocytosis ($\geq$ 100 x 10^9/L in patients with T phenotype or $\geq$ 30 x 10^9/L in patients with B phenotype); *t(9;22) (BCR/ABL)*; *t(4;14),* hypodiploid karyotype and absence of early complete remission (lack of complete remission at day +28 post-induction and the requirement of the next phase of the chemotherapy protocol). Cytogenetic risk was classified in acute myeloid leukemia (AML) patients according to the CALGB (Cancer and Leukemia Group B).[28] *CVC-related thrombosis* was defined as the presence of edema and/or pain at the site of catheter insertion and confirmed by Doppler ultrasound or computed tomography (CT). Due to the retrospective nature of the study, we could only include cases with symptomatic CVC-related thromboses.

Treatment regimens

According to leukemia subtype, several treatment regimens were used in this cohort and included: 7+3;[29,30] HCVAD;[31,32] institutional protocol 0195;[32] ATRA;[33] institutional protocol for mature B-ALL and palliative schemes with single or combination agents (mostly oral). The decision to use one scheme or another was based on clinical evaluation, leukemia subtype, functional status according to the ECOG (Eastern Cooperative Oncology Group) score and socioeconomic level. In secondary leukemia cases or patients in palliative care, treatment was established according to the patient's preference and functional status. L-asparaginase was also incorporated to the treatment in some ALL cases (dose: 10,000 IU/day for 5 to 7 days), mainly in rescue protocols in relapse or refractory disease.

We also registered the starting date of anticoagulant therapy, type of treatment and complications in cases of confirmed thrombosis.

Statistical analysis

Continuous variables were described using medians and ranges and categorical variables with frequencies and proportions. Differences between groups in gender and age were assessed by Fisher's exact test and Mann Whitney's-U test, respectively. We developed Kaplan-Meier curves to asses overall survival of the entire cohort as well as that of leukemia subtypes; differences between them were assessed with a log rank test. In order to identify factors relating to the development of thrombosis, univariate analysis with logistic regression model was conducted. Correlations between variables were established with Pearson's or Spearman's *r* in the SPSS v15.0 statistical platform. P values < 0.05 were considered significant.

RESULTS

General features

We analyzed 181 patients, 80 females (44.2%) and 101 males (55.8%), with a median age of 33 years

(range, 15 to 86 years). The most common subtype of leukemia was ALL (45.8%), followed by AML (39.2%) and APL (10.5%). Six cases (3.3%) were considered secondary acute leukemia. Table 1 shows the general features of the study population.

Table 1. General features of the cohort (n = 181).

	N (%)
• Gender:	
° Male	101 (55.8)
° Female	80 (44.2)
• Age (years), median (range)	33 (15-86)
• Leukemia subtype:	
° AML	71 (39.2)
° ALL:	
B	73 (40.3)
T	5 (2.8)
B mature	5 (2.8)
° APL	19 (10.5)
° MP	8 (4.4)
• Tumor lysis syndrome at diagnosis	9 (4.9)
• Impaired LFTs	11 (6.07)
• Extramedular involvement:	29 (16)
° T	3/29 (10.3)
° B	16/29 (55.1)
° AML	8/29 (27.5)
° APL	1/29 (3.4)
° MP	1/29 (3.4)
• Comorbidity*	77 (42.5)
	Median (range)
• WBC (x10^3):	6.6 (0.16-556)
° Blasts BM (%)	62 (10-99)
° Blasts PB (%)	21 (0-98)
° Promyelocytes BM (%)**	77.5 (4-96)
° Promyelocytes PB (%)**	49 (0-97)
• Platelets (x10^9)	36 (3-791)
• Hemoglobin (g/dL)	8.4 (3.5-16.3)
• LDH (U/L)	335 (38-3212)

*Heart disease, type 2 diabetes mellitus, liver disease, infections (hepatitis C virus, hepatitis B virus, human immunodeficiency virus), other non-haematological malignancies, connective tissue diseases. **In APL cases. BM: bone marrow. PB: peripheral blood. LDH: lactic dehydrogenase. LFTs: liver function tests. AML: acute myeloid leukemia. ALL: acute lymphoid leukemia. APL: acute promyelocytic leukemia. MP: mixed phenotype acute leukemia. T: phenotype T. B: phenotype B.

Analyzable cytogenetic results were obtained in 33.1% (60/181) of cases. In the AML group (APL cases included), 23.3% (14/60) displayed poor prognosis cytogenetics (complex karyotype), 38.3% (23/60) had intermediate risk cytogenetics and 38.3% (23/60), good prognosis cytogenetics. In this latter cytogenetic group, only 6.6% (4/60) were t*(8;21)* positive and 31.6% (19/60) were APL t*(15;17)* positive.

The immunophenotype was available in 148 cases, and 41.2% of these also presented an aberrant marker. We identified 83 patients with ALL of which 26.5% (22/83) met poor prognosis criteria, 5.9% being t *(9;22)* positive.

The median OS of the entire cohort was 349 days (range, 257-440 days), with a median follow-up of 3.5 years. Median OS was not reached in APL patients by the time of this analysis.

Treatment regimens

Of the 181 cases, 97.7% (177/181) received a chemotherapy regimen, 7+3 in 37.8% (67/177), HCVAD in 23.1% (41/177), 0195 institutional protocol in 22% (39/177), mature B ALL protocol in 1.1% (2/177), regimens with fludarabine (FLAG) in 0.56% (1/177), ATRA containing regimens in 9% (16/177) and a palliative treatment option (low-dose cytarabine and/or hydroxyurea) in 6.2% (11/177). Of note, only 11% (20/181) received L-asparaginase as part of the chemotherapy regimen.

Thrombotic events

Fifteen patients in the cohort (8.3%) had an episode of thrombosis of these: DVT was detected in 26.7% (4/15), PE in 6.7% (1/15), acute myocardial infarction (AMI) in 6.7% (1/15), ischemic stroke (IS) in 6.7% (1/15), and CVC-related thrombosis in 53.3% (8/15). In the group with thrombosis, 5 patients had WBC $\geq$ 30 x 10^9/L, none had platelets $\geq$ 450 x 10^9/L and 4 had a D-dimer value > 500 μg/L. The median time to thrombosis development was 92 days (range: 1 to 1,460 days), with 33.3% of cases occurring during the first 30 days after diagnosis. Almost half of the events (46.6%) appeared during the first three months and 93.3% of cases developed the thrombotic event during the first year after diagnosis of acute leukemia. As we previously mentioned, all cases were symptomatic; Doppler ultrasound was the preferred diagnostic tool in 80% of cases, followed by echocardiogram and CT scan in 13.3% and 6.7%, respectively. Only one patient in our study had a PE 4 years after the initial

Table 2. Hemostatic parameters at diagnosis.

	Median	Range	Reference values*
Platelets (x 10^9/L)	36	3-791	150-450
White blood cells (x 10^9/L)	6.6	0.16-556	4-11
Fibrinogen (mg/dL)	432.5	88-1128	238-508
PT (seconds)	11.1	7.7-22.8	9.8-11.1
aPTT (seconds)	29.7	23.4-53.4	24.5-30.8
D-dimer (μg/L)	686.5	50-6570	50-334

PT: prothrombine time. aPTT: activated partial thromboplastine time. *Reference values obtained from the Laboratory of Hemostasis in the Department of Hematology and Oncology, INCMNSZ.

Table 3. Clinical features of patients with thrombosis (n = 15).

Gender	Age (years)	Diagnosis	WBC (x10^9/L) Dg	WBC (x10^9/L) Th	Platelets (x10^9/L) Dg	Platelets (x10^9/L) Th	Type of thrombosis	Time to trombosis (days) †	Related factors
M	76	AML	2.6	-	154	-	DVT	1	T,C,H
F	26	APL	2.3	2.5	66	125	PE	1460* 15**	Qx,C
F	43	ALL (B)	117.7	15.6	5	241	DVT	120	C
F	40	APL	1.6	5.2	22	65	C	30	H,C
F	66	AML	0.5	3.8	83	139	C	150	T,H
F	46	ALL (B)	90.3	5.7	93	352	C	90	C
M	26	AML	140.4	0.5	36	12	C	92	C
F	22	ALL (B)	1.1	5.8	13	170	DVT	30	C
M	22	ALL (B)	247	177	77	86	DVT	150	T,H,C
F	43	AML	5	4	7	258	C	120	T,S,C
M	69	APL	0.7	0.5	17	10	AMI/IS	6	Tp,S,C
M	24	ALL (B)	5.5	5.5	66	126	C	20	T,C,A
F	20	ALL (B)	149	0.5	121	63	C	150	T,S,C,A
M	38	ALL (B)	23	11.6	27	165	AMI/IS	90	T,C,A
F	18	AML	26.6	1.3	9	61	C	150	Tf,S,C

M: male. F: female. AML: acute myeloid leukemia. APL: acute promyelocytic leukemia. ALL: acute lymphoid leukemia. B: phenotype B. DVT: deep vein thrombosis. PE: pulmonary embolism. C: catheter associated thrombosis. AMI/IS: acute myocardial infarction/ischemic stroke. T: smoking habit. H: prolonged hospitalization (≥ 15 days). Qx: previous surgery. S: sedentary (absence of regular physical activity: at least 2 times per week). Tp: history of previous thrombosis. A: L-asparaginase. Tf: history of familial thrombosis. †Days between diagnosis of acute leukemia and thrombosis. Dg: at diagnosis. Th: during the thrombotic event. *Related to previous surgery and during intensive chemotherapy as a second intent of reinduction. **Days since second attempt of induction (detailed information regarding this patient can be found in "Results; Thrombotic events section").

diagnosis of APL, but it is worth mentioning that this event occurred during intensive chemotherapy (day +15) in a second attempt of induction due to relapse and also associated with previous surgery. After excluding this patient from the analysis of time to thrombosis, there was no significant change in the median value (data not shown).

Patients in complete remission who developed DVT and PE, were initially treated with therapeutic doses of low molecular weight heparin (LMWH) or unfractionated heparin (UFH) for the first 5 to 7 days followed by vitamin K antagonists (VKA) (warfarin or acenocoumarol) for up to 6 to 12 months (target INR 2-3). In patients with CVC-related thrombosis, the device was also withdrawn as part of the treatment. Patients with active disease remained on VKA therapy during intensive chemotherapy, as long as there were no contraindications (severe thrombocytopenia or active bleeding). There were no bleeding complications directly linked to antithrombotic therapy.

In patients with arterial thromboses (2 cases), only one patient received anti-platelet therapy with aspirin for a short time and the other pa-

tient died soon after an AMI and could receive neither anti-platelet therapy due to severe thrombocytopenia nor chemotherapy due to his critical clinical condition.

At diagnosis, the following hemostatic parameters were recorded: platelet count ($x10^9$), prothrombin time (seconds), activated partial thromboplastine time (seconds), fibrinogen (mg/dL) and D-dimer (μg/L) (Table 2). Data on fibrinogen and D-dimer were available in only 39.7% cases (72/181). Two patients (2.7%) presented with a fibrinogen value < 100 mg/dL (both with APL) and 43% (31/72) presented with levels above 400 mg/dL. D-dimer values were ≥ 500 μg/L in 59.7% (43/72) of cases. Only 1.1% (2/181) had platelet counts ≥ 450 x 10^9/L, and 26.5% (48/181) had WBC ≥ 30 x 10^9/L.

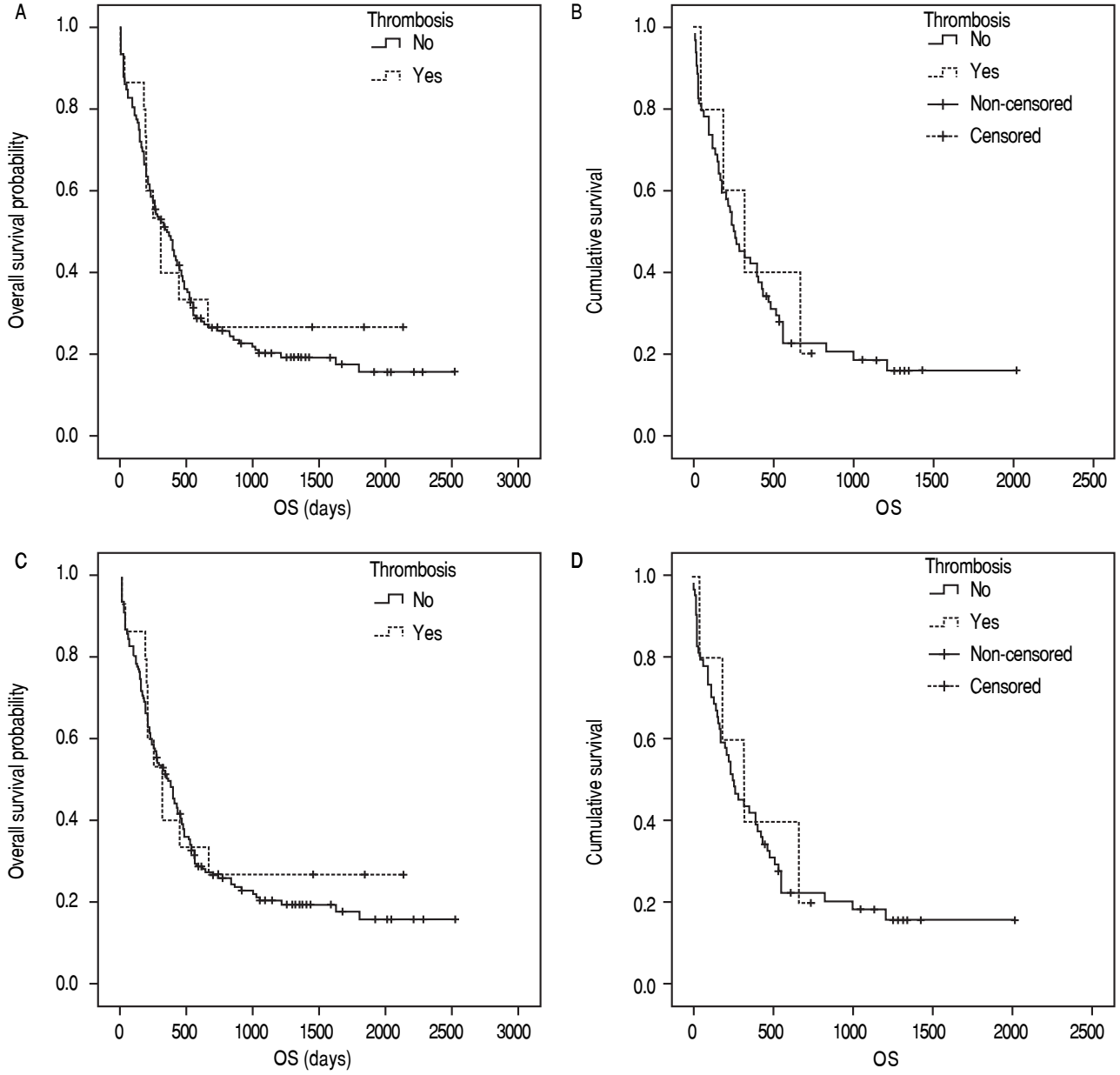

Figure 1. ***A.*** *Overall survival curves of patients with and without thrombosis. Median, 351 days (CI 95%; 254-447 days)* vs. *313 days (CI 95%; 167-458 days); respectively (p = 0.69). B. OS curves of AML patients with and without thrombosis. Median 314 days (CI 95%, range, 30-597 days)* vs. *249 days (CI 95%, range, 146-351 days), respectively (p = 0.81). C. OS curves of ALL patients with and without thrombosis. Median 249 days (CI 95%, range, 120-377 days)* vs. *378 days (CI 95%, range, 265-490 days) (p = 0.62).* ***D.*** *OS curves of APL patients with and without thrombosis. Median not reached in both groups (p = 0.83).*

Of the total cohort, 46.9% (85/181) had a prolonged hospitalization, previous surgery was documented in 1.7% (3/181) and 94.5% (171/181) had required catheter placement. Among catheter types, 50.8% (87/171) were conventional central lines, 36.8% (63/171) were peripherally inserted central catheters (PICC), and 3.5% (6/171) were ports. It is important to point out that due to the high incidence of thrombophlebitis associated to PICC, these devices were withdrawn from our center in October 2007.

As previously stated, only 11% of patients received L-asparaginase, and only 3 cases in this group (15%) had an episode of thrombosis: two CVC-associated thromboses and an AMI, 20, 150 and 90 days after diagnosis, respectively.

At the end of follow-up, 27% (4/15) of patients with thrombosis were alive without evidence of disease and 11 had died, 9 deaths being directly attributable to disease progression. We did not find recurrent episodes of thrombosis, and none of the events had an impact on mortality. Figure 1 shows the survival curves between groups with and without thrombosis. Table 3 describes in detail all data pertaining to the thrombotic events.

Subgroup analysis

An analysis of each leukemia subtype (ALL, AML, APL) was also conducted. General features, incidence of thrombotic events and overall survival were analyzed. The median age at diagnosis was similar in ALL and APL groups, but AML patients were older (43 years) ($p = 0.0001$). In terms of gender, approximately half of all patients with ALL and AML were male whereas the APL group was predominantly female (63.2%, $p = 0.065$). Nevertheless, women with APL did not present more thrombotic events in comparison to men with APL ($p = 0.89$). A third of patients with ALL and AML displayed adverse prognostic factors, and in the latter group, almost half (46.5%) of them had at least one comorbidity at diagnosis. In the group with ALL, the main treatments used were HCVAD (46.3%) and institutional protocol 0195 (47.6%); in the AML group, most patients received the standard 7+3 (87.3%) and 84.21% of patients in the APL group received ATRA as part of the induction regimen. Twenty patients in the ALL group received L-aspa-

Table 4. Subgroup analysis: general features and thrombotic events.

Features	ALL, n = 83	AML, n = 71	APL, n = 19
Age (median, years).	30 (15-86).	43 (15-79).	32 (16-69).
Gender (number, %).	Male 51 (61.4).	Male 36 (50.7).	Male 7 (36.8).
Comorbidity (number, %).	33 (39.8).	33 (46.5).	5 (26.3).
Adverse prognosis (number, %).	22/77 * (28.6).	12/38† (31.6).	NA.
IC (type, number, %).	0195 Protocol 39 (47.6). HCVAD 38 (46.3). B mature protocol 2 (2.4). **L-Asparaginase 20 (24.1).	7+3 62 (87.3). Palliative 9 (12.7).	ATRA 16 (84.2).
TE (incidence) (number, %).	7 (8.4)	5 (7).	3 (15.8).
Type of thrombosis (type, number, %).	DVT 3 (42.8). Catheter 3 (42.8). AMI/IS 1 (14.2).	DVT 1 (20). Catheter 4 (80).	PE 1 (33.3). Catheter 1 (33.3). AMI/IS 1 (33.3).
TtT (days, range).	90 (20-150)	120 (1-150).	30 (6-1460).
Diagnostic tool (type, number, %).	Doppler ultrasound 6 (85). ECO 1 (14.2).	Doppler ultrasound 5 (100).	Doppler 1 (33.3). CT 1 (33.3). ECO 1 (33.3).
OS (days, range).	373 (IC 95% 255-491).	253 (IC 95% 145-361).	NR.

IC: induction chemotherapy. TE: thrombotic events. TtT: time to thrombosis. OS: overall survival. DVT: deep vein thrombosis. PE: pulmonary embolism. AMI/IS: acute myocardial infarction/ischemic stroke. ECO: echocardiography. NA: not applicable. NR: Not reached. *In 6 cases there was no cytogenetic study available. †Cytogenetics was available only in 38 cases. **L-asparaginase was used only in ALL patients as part of rescue treatments at relapse or refractory disease.

raginase as part of rescue treatments at relapse or for refractory disease. Interestingly, only 15% (3/20) also developed a thrombotic complication but there was no statistically significant association between the presence of thrombosis and the use of this particular drug (p = 0.21). There were no statistically significant differences in the incidence of thrombotic events among patients with ALL, AML or APL (8.4% *vs.* 7% *vs.* 15.8%; respectively, p = 0.78). CVC-related thrombosis was the most frequent type of thrombotic event in ALL and AML patients (42.8 and 80% respectively), and there was only one case of PE in the group with APL. The median time to thrombosis was 90, 120 and 30 days for ALL, AML and APL patients, respectively. Median OS (days) for ALL and AML was 373 and 253 days, while in APL patients, OS was not reached by the time of this analysis. In patients with AML, the median OS was 314 days *vs.* 249 days when comparing cases with and without associated thrombotic events (p = 0.81). In patients with ALL, the median value was 249 days *vs.* 378 days, respectively (p = 0.62) and was not reached in APL patients. These results are shown in detail in table 4, figure 1. In order to identify variables relating to the development of thrombosis, we performed an univariate analysis between thrombosis and each of the following: gender, age, chemotherapy regimen, WBC count, platelet count, smoking habit, sedentary, previous surgery, fibrinogen, D-dimer and presence of a CVC. We did not identify any specific variable associated with thrombosis development and therefore a multivariate analysis was not done.

DISCUSSION

This study reveals an incidence of thrombosis of 8.3% for the entire group, placing it within the range reported by others[10,11,13,26,34] and without significant differences between leukemia subtypes. The study by Ziegler, *et al.,* a retrospective analysis of 719 adult patients with acute leukemia, showed a lower incidence of venous thromboembolism (2%). But concordant with our results, these authors also failed to find statistically significant differences among leukemia subgroups.[10] On the other hand, Stefano, *et al.,* found a 6.3% incidence of thrombosis in 379 adult patients; this incidence was higher in patients with APL and in those receiving L-asparaginase (increased risk: 4.9 fold), with a thrombosis-associated mortality rate of 0.8%.[13] Although we observed an incidence of thrombosis of 15.8% in APL patients, we could not demonstrate statistically significant differences in comparison to patients with ALL or AML. In terms of gender, only in the APL group did we find a female predominance (p = 0.065). We must consider the small sample size for each leukemia subtype in this study, precluding reliable conclusions regarding age and gender.

CVC-related thrombosis was the most common subtype of thrombotic event, accounting for 53.3% of all cases. However, the incidence among the whole cohort was only 4.4%, a rate placed between that reported in most studies, which ranges from 0.3 to 30%.[35]

Variations in the incidence of thrombosis depend on the treatment regimen and study design (prospective *vs.* retrospective) whereby prospective studies tend to report higher incidences of thrombotic events. On the other hand, studies designed to detect asymptomatic thrombosis have also reported increased thrombosis incidence rates.[36-38] As previously mentioned (see *definitions* in *Material and methods*), this study was designed to detect only symptomatic thromboses and may therefore underestimate the real incidence of these phenomena.

The thrombogenic role of L-asparaginase in patients with ALL, especially in pediatric patients, has been well demonstrated;[22] however, this tendency is less consistent in the adult population. In a retrospective study of 214 ALL-adult patients receiving induction chemotherapy containing L-asparaginase, an incidence of thrombosis of 9.8% was reported.[39] In our cohort, 11% of patients received L-asparaginase and only in 3 cases (15%) was an episode of thrombosis recorded. However, despite a higher frequency of thrombosis in patients receiving L-asparaginase in comparison to the rest of ALL patients, this difference was not statistically significant (p = 0.21). These results should be taken cautiously due to the small number of cases in these groups.

Other factors associated with an increased risk of thrombosis in cancer patients have been previously described by different study groups. Among the most important are: leukocytosis, thrombocytosis,[2,3,40] older age, number of comorbidities, presence of intravenous catheters,[12] high levels of D-dimer,[41] low fibrinogen level (< 170 mg/dL), variant APL morphology,[42] inherited thrombophilia,[43] expression of CD15 and CD2, as well as the short isoform of PML/RARα in APL patients.[44] Nevertheless, the heterogeneity and lack of standardization of these variables, especially in adult acute leukemia patients, does not allow their use on a routine basis in order to predict the development of thrombosis; further research in larger cohorts is required. In this study, we did not identify any particular variable relating to the deve-

lopment of thrombosis in association with each leukemia subtype. This could also be explained by the aforementioned study limitations (design, sample size) and provides a rationale for prospective studies with a larger number of patients.

The median time to thrombosis in this cohort was 92 days (range 1-1,460 days) and almost half of the events occurred during the first three months after the diagnosis of acute leukemia, but no statistically significant differences between leukemia subtypes were detected ($p = 0.66$). These results are similar to the data obtained by Ku, *et al*., in a population-based cohort of 5,394 patients with AML, with 64% of thrombotic events developing within the first 3 months of leukemia diagnosis.[12] In the present study, only 33% of thrombotic events occurred in the first month but were never the initial manifestation of the neoplastic disease. This is different to what has been reported in the study of Melillo, *et al*., a prospective analysis of 114 adult patients in which most of the events occurred at diagnosis or during induction chemotherapy;[14] in De Stefano's study, thrombosis was the initial manifestation in 3.4% of cases.[13]

This timing pattern is different in pediatric populations. In children, most of the events occur during the first month of diagnosis, most of them during induction chemotherapy, and some of them are associated to other risk factors such as L-asparaginase and steroid use in current protocols.[45] In our study 90% of thromboses occurred during the first year after the acute leukemia diagnosis. Therefore, obtaining data on VTE timing in adult leukemia patients will increase clinician's awareness of these complications over a longer period during the disease's course and not only during the intensive induction phase.

It has recently been reported that thromboembolic complications have a negative impact on survival in patients with cancer, as demonstrated in the study by Sorensen. *et al.,* where one-year OS decreased in patients with thrombosis (12%) when compared to 36% of those without thrombotic complications ($p = 0.001$).[46,47] Nevertheless, differences on thrombosis impact on mortality when comparing AML and ALL patients have been reported by Ku, *et al.*[12] These authors have demonstrated that as opposed to AML patients in whom survival was not affected by VTE, ALL patients showed a 40% increase in the risk of death during the first year after thrombosis. In our study, thrombosis did not affect OS, not even in the subgroup analysis, similar results to what Ramos, *et al*. reported in a cohort of 153 ALL-patients.[26]

Finally, we must acknowledge the strengths and limitations of this study. Its strengths are underscored by the fact that this cohort contributes to the limited information on thrombosis in Latin American adults with acute leukemia and that all events were documented with objective methods. As far as we know, there is only one previous report of VTE in adult Mexican patients with ALL,[26] and our study would be the first to estimate the incidence of thrombotic events, analyze the clinical characteristics and survival in different leukemia subtypes in an adult population in Mexico. Its main limitations include the retrospective nature of the cohort and the small number of thromboses cases detected; this did not permit the identification of risk factors associated with thrombosis. Since only symptomatic patients were included, we may have missed cases of asymptomatic thrombosis. Despite these restrictions, this study provides an excellent rationale to develop future prospective studies addressing these issues in adult acute leukemia patients.

CONCLUSIONS

The present study shows an incidence of thrombosis in this adult acute-leukemia population in Mexico comparable to that reported in the international literature. Of note, only a third of cases occurred during the first month of diagnosis and almost half of the events developed during the first three months. All cases were symptomatic and CVC-related thrombosis was the most frequent event. Survival curves were similar between patients with and without thrombosis. Larger prospective studies are needed to identify risk factors for thrombosis development in adult patients with acute leukemia.

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