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**Original Article**

# Baseline Thrombin Generation Test Does Not Predict Thrombotic Events in Acute Leukemia: A Monocentric Prospective Study

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**Abstract. Thromboembolic and hemorrhagic complications are significant causes of morbidity and mortality in patients with acute leukemias (AL). While AL is characterized by a complex hemostatic imbalance, conventional coagulation tests and platelet counts offer limited predictive value for bleeding and thrombotic events. Global coagulation assays (GCAs), such as the Thrombin Generation Assay (TGA), provide a more comprehensive assessment of coagulation potential and may offer improved risk stratification. This prospective, single-center pilot study aimed to explore the utility of TGA in newly diagnosed adult patients with AL. Between February 2022 and September 2024, 111 patients were enrolled at the Department of Translational and Precision Medicine, Sapienza University of Rome. Baseline clinical and laboratory data, including TGA parameters, were collected, and patients were monitored for thrombotic events until death or last follow-up. TGA values at diagnosis displayed wide inter-individual and inter-subtype variability. With a median follow-up of 8.28 months, 8 (7.2%) thrombotic events were reported. No statistically significant association was found between baseline TGA parameters and the development of thrombotic events ($p > 0.05$). These findings suggest that a single TGA measurement at diagnosis may not predict thrombotic risk in AL patients. Future studies incorporating longitudinal TGA assessments and additional hemostatic evaluations, such as platelet function analysis, may help refine risk prediction for both thrombotic and hemorrhagic complications in this high-risk population.**

**Keywords:** Global Coagulation Assays; Thrombin Generation Assay; Haemostasis; Thrombosis; Anticoagulation; Acute Leukemia.

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**Introduction.** Venous thromboembolism (VTE) and bleeding are important causes of morbidity and mortality in oncologic patients, especially in the onco-hematologic setting. In this population, the incidence of VTE ranges from 2.5-9.3% and the incidence of bleeding can reach 20-51.3%, depending on patient, disease, and treatment-related factors.[1-6]

Conventional coagulation assays (CCAs) and platelet count (Plts) provide minimal information on the hemostatic process and are not good predictors of bleeding and thrombotic risk. Global coagulation assays (GCAs), which allow a comprehensive analysis of the

entire hemostatic process, may provide more information. However, these tests are not routinely performed.

Recently, Raso et al.[7] have shown the complexity of hemostatic alterations in acute myeloid leukemia (AML) through thromboelastography (TEG), a GCA that measures the viscoelastic properties of the clot, providing information from the clot formation to fibrinolysis.[8] However, further evidence is required to determine its potential usefulness in this context.

Another promising test is represented by the thrombin generation assay (TGA), which evaluates the formation and decay of thrombin upon activation of the coagulation cascade by tissue factor (TF).[9] Recently, Betticher et al.[10] published their experience about the application of TGA in a pediatric cohort diagnosed with acute lymphoblastic leukemia (ALL), reporting that this assay, evaluated during induction treatment, is a predictor of thrombotic complications. However, additional research is necessary to clarify its role in this setting.

In our center, a prospective observational monocentric pilot study was performed to evaluate the possible association between TGA parameters at diagnosis and thrombotic risk during follow-up in patients with acute leukemia (AL).

**Materials and Methods.**

*Study population.* Eligible patients were subjects ≥ 18 years of age diagnosed with AML, acute promyelocytic leukemia (APL), mixed phenotype acute leukemia (MPAL), and ALL as per international guidelines[11-14] diagnosed between February 2022 and September 2024 in the Department of Translational and Precision Medicine of Sapienza University of Rome. Anticoagulant and antiplatelet therapy at the time of AL diagnosis constituted an exclusion criterion. All enrolled subjects provided informed consent according to the principles of the Declaration of Helsinki.

*Clinical characteristics and data collection.* Baseline characteristics, including age, sex, AL subtype, previous history of VTE, and laboratory parameters, were systematically recorded in a secure, study-specific database accessible exclusively to authorized study personnel. Patients were prospectively followed for the occurrence of thrombotic events until the last follow-up or death from any cause. Thrombotic events were defined as VTE, superficial venous thrombosis (SVT), or arterial thrombosis. Suspected venous thrombotic events were confirmed by appropriate imaging modalities, including Doppler ultrasound and/or contrast-enhanced computed tomography with iodinated contrast media. Arterial thrombotic events were confirmed using computed tomography angiography.

*Laboratory evaluation.* At the time of AL diagnosis, a baseline assessment of complete blood counts (CBCs), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fg), anti-thrombin (AT), and TGA was performed. Blood samples were collected using either a 19–21-gauge needle or a central venous catheter (CVC), with the first 5 mL discarded; then they were analyzed within 2 hours after collection. Coagulation samples were collected in citrated blood [anticoagulated with $10^9$ mmol/l (3.2%) trisodium citrate] in the absence of any concomitant anticoagulant treatment.

Thrombin generation assay. The thrombin generation assay, a global coagulation test that reproduces the kinetics of thrombin formation, was performed using the Calibrated Automated Thrombogram (CAT) (Diagnostica Stago, Asnières, France) according to the manufacturer's specifications. Twenty-two plasma samples *per plate* were mixed with assay reagents (tissue factor and phospholipids), and the fluorescent signal indicating TG was monitored in a Fluoroskan Ascent Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Parameters were calculated with the Thrombinoscope Software Program (Thrombinoscope BV, Maastricht, The Netherlands). The following parameters were included: thrombin peak, which represents the maximum concentration of thrombin formed at any time; endogenous thrombin potential (ETP), which depicts the total amount of thrombin generated over time and reflects the total enzymatic activity of thrombin; time to peak (ttPeak), which indicates the time required to reach the thrombin peak; the lag time, which measures the time between the start of the assay and the initial formation of TG; the velocity index, which is defined as (peak height/[time to peak-lag time]) indicating the rate of TG formation.

TGA parameters' normal values were established based on the analysis of pooled normal plasma (**Table 1**).

Complete blood count and conventional coagulation assays. Complete blood count was performed using an ADVIA 2120 analyzer (Siemens, Munich, Germany);

PT, APTT, and AT were performed using the automated coagulometer BCS Xp (Siemens), and Fg was assayed by the Clauss method using reagents and methodology according to the manufacturer's instructions.

All blood samples were collected and processed within 2 hours. The tubes were centrifuged for 10 minutes at 4500 rpm.

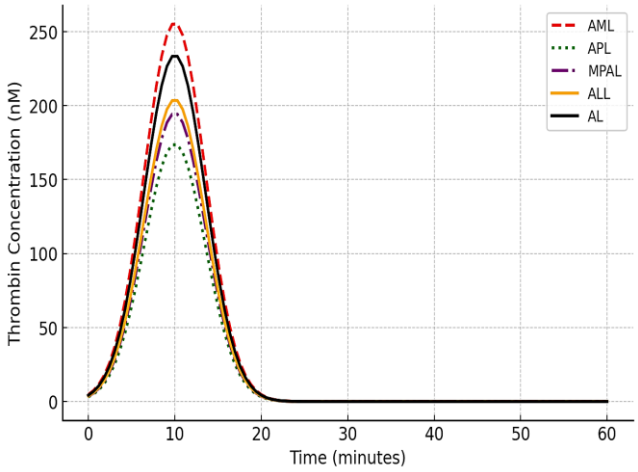
*Statistics.* We performed a descriptive analysis of our case series, including continuous variables expressed in terms of median and range, and categorical variables expressed as frequencies. Overall survival (OS) has been defined as the time from the AL diagnosis to the last follow-up or death for any cause. Event-free survival

**Table 1.** TGA patients' values.

| TGA parameters | Patients' values, Median (Range) | Patients with increased values (%) |
|---|---|---|
| Lag Time (N.V. ≤ 2.42 min)* | 4.1 min (2-11.83) | 111 (90.9) |
| ETP (N.V. ≤ 1249.63 nM x min)* | 1200.44 nM/min (465.59-2412.94) | 70 (63.1) |
| Peak (N.V. ≤ 227.73 nM)* | 234.11 nM (59.18-399.51) | 58 (52.3) |
| ttPeak (N.V. ≤ 5.84 min)* | 7 min (4.17-15.5) | 74 (66.7) |

N.V.: Normal Values. *Based on pooled normal plasma.

**Figure 1.** TGA curves in patients with acute leukemia.



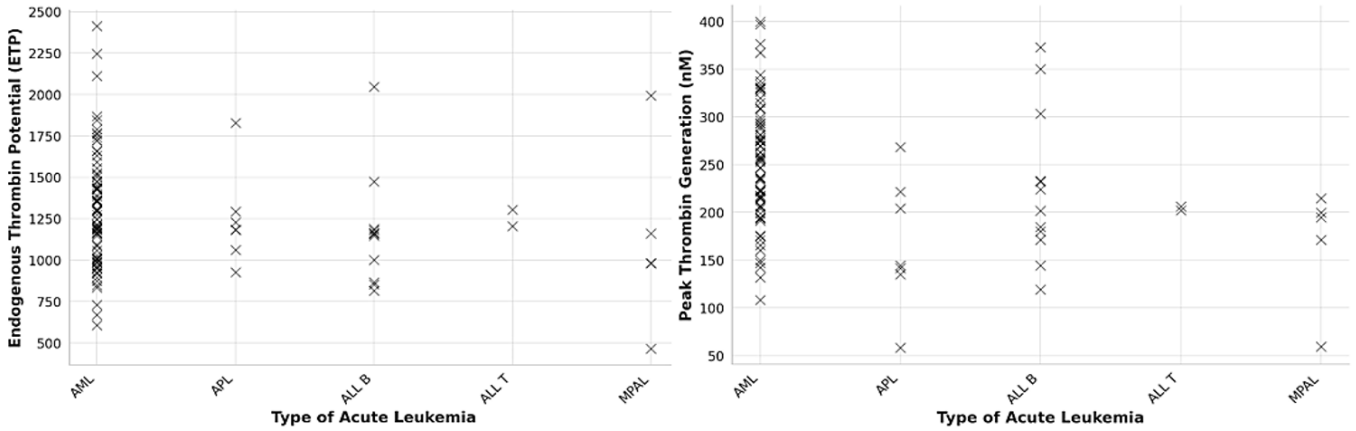

Curves were generated based on median TGA parameters. There were no statistically significant differences in Lag Time (p=0.40), ttPeak (p=0.33), ETP (p=0.15), and peak (p=0.53) among different types of AL. AML: Acute Myeloid Leukemia (APL excluded), APL: Acute Promyelocytic Leukemia, MPAL: Mixed Phenotype Acute Leukemia, ALL: Acute Lymphoblastic Leukemia, AL: Acute Leukemia.

(EFS) was calculated as the time from the AL diagnosis to the onset of adverse thrombotic events (T-AEs), when they occurred. The cumulative incidence of thrombosis was depicted with the 1-KM method. Chi-square analysis and Mann-Whitney U test were performed to evaluate the differences between subgroups. Cox regression was performed to evaluate the association between the development of thrombotic events, TGA parameters, and other laboratory values, when appropriate. Statistical significance was considered for values of $p < 0.05$. Statistical analysis was performed with the "IBM SPSS Statistics" software version 26.

## Results.

*Patients' baseline characteristics and AL treatment.* Patients' baseline characteristics are summarized in **Table 2**. One hundred and eleven patients were enrolled: 58 (52.3%) were females and 53 (47.7%) were males. Two patients (1.8%) experienced an episode of VTE before the diagnosis of AL. Median age at AL diagnosis was 63.6 years (range 18-86.4). Eighty-three patients (74.8%) were diagnosed with AML (APL-excluded), 15 (13.5%) with ALL, 8 (7.2%) with APL, and 5 (4.5%) with MPAL. At diagnosis, CVC was placed in 63 patients (56.8%); median bone marrow blast count was 60% (range 10-100%); median CBC values were hemoglobin 9.5 g/dL (range 4.5-14.6), white blood cells $6.03*10^3$/mmc (range 0.44-116.7), platelets $66*10^3$/mmc (range 3-361). Median values of CCAs were PT ratio 1.06 (range 0.8-3.32), APTT ratio 0.96 (range 0.73-1.6), Fg 363 mg/dL (range 54-1026), AT 94.5% (range 55-126). Thrombin generation assay median parameters were Lag Time 4.1 min (range 2-11.83), ETP 1200.44

**Figure 2.** Variability in ETP and Peak in AL at baseline.




AML: Acute Myeloid Leukemia (APL excluded), APL: Acute Promyelocytic Leukemia, MPAL: Mixed Phenotype Acute Leukemia, ALL: Acute Lymphoblastic Leukemia, AL: Acute Leukemia.

**Table 2.** Patients' Characteristics and Thrombotic Events.

| | Sex, M/F | Age (years), Median (Range) | Risk [11-14], N (%) | Bone marrow blasts (%), Median (Range) | HB (g/dl), Median (Range) | WBC*$10^3$/mmc, Median (Range) | PLT*$10^3$/mmc, Median (Range) | Lag time (min), Median (Range) (N.V. < 2.42 min)† | ETP (nM/min), Median (Range) (N. V. < 1249.63 nM x min)† | Peak (nM), Median (Range) (N. V. < 227.73 nM)† | ttPeak (min), Median (Range) (N. V. < 5.84 min)† | Follow up, months, Median (Range) | Thrombotic events, N (%) | Deaths, N (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AL (N = 111)** | 53/58 | 63.6 (18.0-86.1) | Evaluated per subgroups (different criteria) | 60 (10-100) | 9.5 (4.5-14.6) | 6.0 (0.4-116.7) | 66.0 (3.0-361.0) | 4.1 (2-11.83) | 1200.44 (465.59-2412.94) | 234.11 (59.18-399.51) | 7 (4.17-15.5) | 8.28 (0.03-32.4) | 8 (7.20) | 32 (28.8) |
| **AML (N =83)*** | 39/44 | 64.7 (19.8-88.7) | Low: 7 (8.4) Intermediate: 63 (75.9) High: 13 (15.7) | 50 (10-96) | 9.4 (4.5-14.6) | 6.1 (0.4-116.7) | 63.0 (3.0-361.0) | 4.05 (2.17-9) | 1260.59 (608.31-2412.94) | 255.86 (107.99-399.51) | 6.83 (4.17-12.33) | 7.8 (0.03-32.4) | 5 (6) | 25 (30) |
| **APL (N = 8)** | 3/5 | 56.7 (23.4-67.6) | Low: 8 (100) | 86 (53-91) | 11.6 (9.4-13.9) | 2.4 (0.9-9.4) | 64.0 (5.0-120.0) | 4.83 (3.33-8.67) | 1183.41 (928.11-1830.04) | 174.08 (135.09-268.28) | 8.5 (7-12) | 15.55 (3.8-28.7) | 2 (25) | 0 |
| **MPAL (N = 5)** | 4/1 | 63.4 (56.6-76.4) | N.E. | 82 (65-90) | 9.1 (7.1-10) | 72.1 (2.4-81.3) | 130.0 (81.0-357.0) | 4.83 (4-9.67) | 982.88 (465.59-1994.06) | 194.82 (59.18-214.66) | 8.5 (5.33-13) | 10.2 (0.13-30.0) | 1 (20) | 3 (60) |
| **ALL (N = 15)** | 7/8 | 53.5 (18.0-86.1) | Standard: 4 (26.7) High: 4 (26.7) Very high: 7 (46.6) | 83 (25-100) | 10 (8.2-14.1) | 6.4 (1.5-69.5) | 66.0 (28.0-313.0) | 3.67 (2-11.83) | 1162.57 (853.84-2047.49) | 204.08 (119.11-372.69) | 6.92 (4.33-8.83) | 9.33 (1.0-31.8) | 0 | 4 (26.7) |

M: Male, F: Female, HB: Hemoglobin, WBC: White Blood Cells, PLT: Platelets, N.V.: Normal Values, ETP: Endogenous Thrombin Potential, ttPeak: time to Peak, AL: Acute Leukemia, AML: Acute Myeloid Leukemia, APL: Acute Promyelocytic Leukemia, MPAL: Mixed Phenotype Acute Leukemia, ALL: Acute Lymphoblastic Leukemia, N.E.: Not Evaluable. *APL excluded. †Based on pooled normal plasma.

**Table 3.** AL treatment.

| Patients (N = 111) | Therapy based on intention to treat (N, %) |
|---|---|
| AML* (N = 83) | Supportive care† (11, 9.9)<br>Hydroxyurea (4, 3.6)<br>IC±Immunotherapy+allo-SCT (45, 40.5)<br>Hypometilating agent ± Venetoclax (23, 20.7) |
| APL (N = 8) | ATRA+ATO (8, 7.2) |
| B-/T-ALL (N = 15) | Supportive care† (2, 1.8)<br>Immunotherapy ± allo-SCT (2, 1.8)<br>IC ± allo-SCT (9, 8.1)<br>LC+Immunotherapy (2, 1.8) |
| MPAL (N = 5) | IC±Immunotherapy+allo-SCT (3, 2.7)<br>Decitabine-Venetoclax (1, 0.9)<br>Hydroxyurea (1, 0.9) |

AML: Acute Myeloid Leukemia, APL: Acute Promyelocytic Leukemia, ALL: Acute Lymphoblastic Leukemia, MPAL: Mixed-Phenotype Acute Leukemia, IC: Intensive Chemotherapy, LC: Low-dose Chemotherapy, Allo-SCT: Allogeneic Hematopoietic Stem Cells Transplantation. *APL-excluded. †Cytoreduction-excluded.

nM x min (range 465.59-2412.94), Peak 234.11 nM (range 59.18-399.51), ttPeak 7 min (range 4.17-15.5) (**Figure 1-2**). Median follow-up was 8.28 months (range 0.03-32.4).

One hundred and one patients (90.9%) presented an increase in Lag Time, 74 (66.7%) in ttPeak, 70 (63.1%) in ETP, 58 (52.3%) in peak.

There were no statistically significant differences in Lag Time (p=0.40), ttPeak (p=0.33), ETP (p=0.15), and peak (p=0.53) among different types of AL.

AL treatment is reported in **Table 3**.

*Thrombotic events.* Eight patients (7.2%) experienced thrombotic events: 4 (3.6%) deep venous thrombosis (DVT) and 4 (3.6%) CVC-related thrombosis. All eight patients had a CVC at the time of thrombosis. Two patients had a history of previous VTE. Five (4.5%) were diagnosed with AML (APL-excluded), 2 (1.8%) by APL, and 1 (0.9%) by Philadelphia-positive-MAPL. Time from diagnosis to thrombosis was 0.13 months (range 0-4.07).

All eight patients presented an increase in Lag Time (100%), 6 in ttPeak (75%), 4 in peak (50%), and 1 (12.5%) in ETP (**Figure 3**).

No statistically significant differences were observed in Lag Time (p=0.60), ttPeak (p=0.57), ETP (p=0.56), or Peak (p=0.69) between patients who developed thrombosis and those who did not. Similarly, no significant differences in thrombotic events were found across different types of AL (p=0.23) or treatment (p=0.62). Baseline platelet count (p=0.33), LDH (p=0.35), and hematocrit (p=0.96) were also not predictive of thrombosis development. In contrast, a statistically significant association was found between a history of previous VTE and the occurrence of thrombosis (p=0.03).

The cumulative incidence of thrombotic events is shown in **Figure 4**.

Management of thrombotic events. Two thrombotic events occurred at the onset of acute leukemia, while six

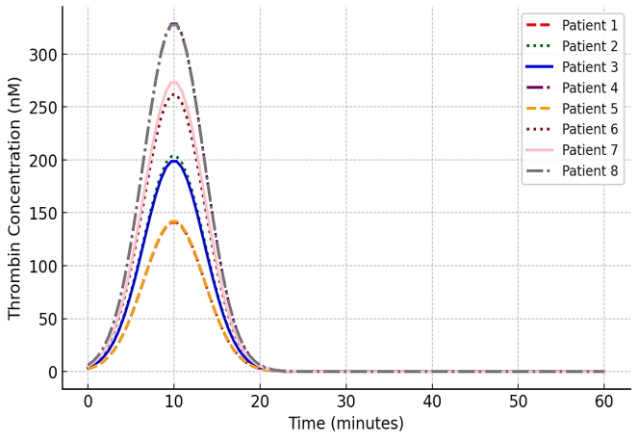



**Figure 3.** TGA in patients with acute leukemia who experienced thrombosis. No statistically significant differences were observed in Lag Time (p=0.60), ttPeak (p=0.57), ETP (p=0.56), and Peak (p=0.69) between patients who developed and those who did not develop thrombosis.

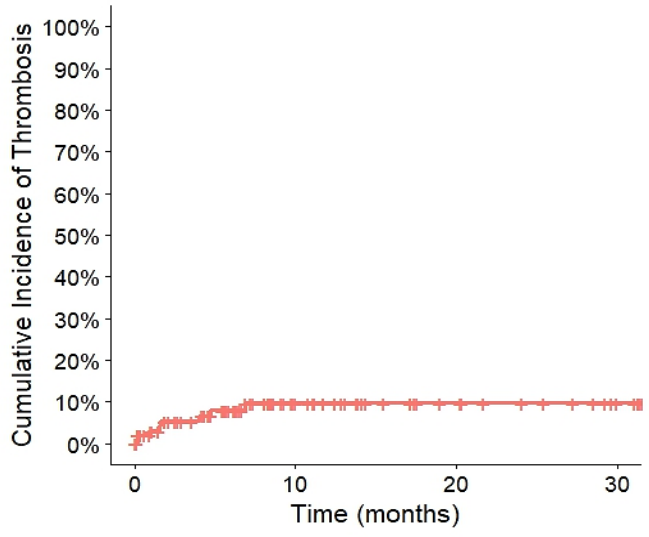



**Figure 4.** Cumulative incidence of thrombosis.

**Table 4.** Baseline characteristics of patients who experienced a thrombotic event and their management.

| | Sex | Age, years | Leukemia characteristics [11-14] | HB (g/dl) | WBC*$10^3$/mmc | PLT*$10^3$/mmc | Lag time (min), Median (Range) (N.V. < 2.42 min)† | ETP (nM x min), Median (Range) (N. V. < 1249.63 nM/min)† | Peak (nM), Median (Range) (N. V. < 227.73 nM)† | ttPeak (min), Median (Range) (N. V. < 5.84 min)† | Time to thrombosis (months) | Status of Leukemia at Thrombosis | Treatment for Leukemia at Thrombosis | Type of thrombosis | Management |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Patient 1** | F | 49 | Low risk APL | 11.7 | 1.8 | 91.0 | 3.33 | 928.11 | 141.43 | 7.17 | 0 | Onset | None | Splanchnic | Enoxaparin 100 IU/Kg x2/day for 3 months ➔ secondary prophylaxis with Apixaban 2.5 mg x2/day |
| **Patient 2** | F | 67 | Low risk APL | 13.9 | 1.4 | 87.0 | 4.67 | 1183.41 | 203.87 | 8.17 | 1 | CR | ATRA+ATO | DVT+PE | Enoxaparin 100 IU/Kg x2/day for 7 days ➔ Rivaroxaban 20 mg/day for 6 months ➔ secondary prophylaxis with Rivaroxaban 10 mg/day |
| **Patient 3** | M | 63 | Ph+ MPAL | 7.1 | 81.3 | 357.0 | 2.67 | 982.88 | 199.35 | 5.33 | 6.83 | CR | Allogeneic HSCT | CVC-related | Enoxaparin 6000 IU/day for 7 days (withhold for thrombocytopenia) |
| **Patient 4** | F | 42 | Low risk AML | 9.6 | 24.0 | 4.0 | 4.33 | 1844.72 | 329.6 | 7 | 1.53 | CR | None | CVC-related | Enoxaparin 4000 IU/day for 14 days (withhold for thrombocytopenia) |
| **Patient 5** | F | 59 | Intermediate risk AML | 11.3 | 116.7 | 44.0 | 7.17 | 919.38 | 142.25 | 10.67 | 1.63 | CR | HDAC+ Midostaurin | CVC-related | Enoxaparin 6000 IU/day for 45 days |
| **Patient 6** | F | 65 | Intermediate risk AML | 8.5 | 1.2 | 87.0 | 3.83 | 1247.16 | 262.52 | 6.5 | 4.77 | CR | None | CVC-related | Tinzaparin 10000 IU/day for a week ➔ PICC removal |
| **Patient 7** | M | 47 | Intermediate risk AML | 9.5 | 108.6 | 19.0 | 5.17 | 1185.07 | 274.08 | 7.5 | 0.13 | Onset | HU | DVT | No anticoagulation for thrombocytopenia<br>Death during induction for progressive disease |
| **Patient 8** | M | 77 | High risk AML | 9.6 | 24.0 | 4.0 | 2.93 | 1660.47 | 328.75 | 5.1 | 4.07 | CR | Azacitidine+ Venetoclax | DVT | Tinzaparin 14000 IU/day (ongoing) |

M: Male, F: Female, HB: Hemoglobin, WBC: White Blood Count, PLT: Platelet Count, N.V. Normal Values, ETP: Endogenous Thrombin Potential, ttPeak: time to Peak, APL: Acute Promyelocytic Leukemia, MPAL: Mixed Phenotype Acute Leukemia, Ph+: Philadelphia-positive, AML: Acute Myeloid Leukemia, CR: Complete Response, ATRA: All-Trans Retinoic Acid, ATO: Arsenic Trioxide, HSCT: Hematopoietic Stem Cells Transplantation, DVT: Deep Venous Thrombosis, PE: Pulmonary Embolism, SVT: Superficial Venous Thrombosis, CVC: Central Venous Catheter, HDAC: High Dose Cytarabine, HU: Hydroxyurea.
†Based on pooled normal plasma.

events developed in patients who were in complete remission.

At disease onset, one patient with low-risk acute promyelocytic leukemia (APL) was diagnosed with splanchnic vein thrombosis and was treated with enoxaparin 100 IU/kg twice daily for three months, achieving complete resolution; the patient was subsequently switched to secondary prophylaxis with apixaban 2.5 mg twice daily. Another patient with intermediate-risk acute myeloid leukemia (AML) developed deep vein thrombosis (DVT) of the lower limbs at diagnosis; anticoagulation was not feasible due to severe thrombocytopenia, and the patient died during induction therapy from progressive disease.

All other thrombotic events occurred after patients had achieved complete remission and were managed with low molecular weight heparin (LMWH), specifically enoxaparin and tinzaparin. Four events were catheter-related: one patient received a full 45-day course of enoxaparin at 100 IU/kg/day, while the remaining three received enoxaparin at 100 IU/kg/day or tinzaparin at prophylactic doses for 7–14 days. In three cases, anticoagulation was interrupted due to thrombocytopenia, with the central venous catheter retained as clinically necessary; in one case, anticoagulation was discontinued after CVC removal. The remaining patients developed lower-limb DVT, one of which was complicated by pulmonary embolism. One patient received tinzaparin at a therapeutic dose, with treatment ongoing during the acute phase, whereas another patient received rivaroxaban 20 mg daily for six months, followed by secondary prophylaxis after DVT resolution.

Management of thrombotic events is summarized in **Table 4**.

**Discussion.** Thrombotic complications are a frequent and clinically significant problem in patients with AL. These complications arise from a multifactorial pathophysiological environment, shaped by several factors such as underlying malignancy, antineoplastic treatment, infections, and CVC placement.

The current guidelines for the prevention and management of hemostatic complications in AL are based on CCAs and platelet count, which guide the implementation of antihemorrhagic strategies and anticoagulant dosing in patients with thrombotic complications.[15] However, these parameters provide only a partial view of the hemostatic balance, leading to a growing interest in GCAs for a more comprehensive hemostatic assessment.

In our prospective observational study, we investigated the potential utility of TGA in predicting thrombotic events in a large monocentric cohort of newly diagnosed AL patients. While over 60% of patients exhibited elevated ETP and more than half had increased Thrombin Peak levels at diagnosis, consistent with a hypercoagulable state, only 7.2% of the cohort developed thrombotic events during follow-up. No statistically significant association was observed between baseline TGA parameters and the occurrence of thrombosis.

This discrepancy between laboratory hypercoagulability and thrombotic complications suggests that TG alone may not be sufficient to predict thrombosis risk in AL. Indeed, hemostasis in leukemia is profoundly influenced by dynamic and interacting factors. The leukemic microenvironment, for example, is known to induce endothelial activation, inflammatory cytokine release, and microparticle formation, which may promote coagulation independently of thrombin kinetics.[16]

We evaluated TGA parameters at baseline. However, as discussed above, hemostatic parameters undergo changes throughout the disease's progression. Betticher et al.[10] reported that TGA parameters at baseline do not predict the development of thrombotic complications in pediatric ALL; however, ETP values at 8-12 days from the start of induction therapy were predictive of thrombotic complications ($p < 0.05$). Consistently, Rozen et al.[17-18] found that treatment during the induction phase and late intensification in pediatric ALL leads to an increase in ETP and peak levels. Furthermore, native asparaginase leads to a more marked increase in enzyme activity than peg-asparaginase. However, this finding is in contrast with the higher incidence of thrombosis with peg-asparaginase vs native asparaginase during induction ($p = 0.0035$) and late intensification ($p = 0.0096$).[19]

These experiences suggest that a dynamic evaluation of TG parameters may be useful for predicting thrombotic complications in the setting of ALL. No data is available on AML. However, Raso et al.[7] failed to demonstrate an association between TG, indirectly calculated with a TEG algorithm at three timepoints (diagnosis, during the first cycle of chemotherapy, at the end of chemotherapy), and thrombotic or hemorrhagic complications.

Another interesting finding in our experience is the consistent increase in Lag Time across our cohort. While it is true that a shortened Lag Time is associated with hypercoagulability,[20] several hypotheses may explain the paradox of an increased Lag Time in a hypercoagulable state. Lag time is prevalently determined by levels of tissue factor pathway inhibitor (TFPI), protein S (PS), factor VII (FVII), FIX, and fibrinogen. Increased levels of TFPI, an endogenous Kunitz-type serine protease inhibitor expressed by endothelial cells and platelets that inhibits both the TF/FVIIa complex and early forms of prothrombinase, could explain an increase in lag time. It is known that in APL there is an increase in TFPI levels, but they seem to be normal in other AMLs and ALLs.[21-

[24] However, data regarding TFPI levels in AMLs and ALLs is based on small numbers of patients. Another possible explanation could be the binding of the initial traces of thrombin to fibrinogen/fibrin, which hinders the feedback activation of upstream coagulation factors by thrombin itself.[25] In our cohort, the median fibrinogen levels were normal (363 mg/dl). However, we did not evaluate TFPI, PS, FVII, and FIX levels, limiting further analysis. Despite the increased lag time, unquestionably, the higher ETP and Peak values indicate a hypercoagulable state.

Interestingly, all thrombotic events in our cohort occurred in patients with a CVC, reinforcing the established role of CVCs as a risk factor for venous thromboembolism[26-27] and the relevance of non-leukemia-associated factors in the evaluation of thrombotic risk in this setting. Another notable finding was the association between thrombotic events after the diagnosis of AL and a history of VTE (p=0.03). Although our analysis is limited by the small sample size and low number of events, this result is consistent with previous reports in the literature.[28–29]

In our analysis, we also evaluated the possible association between platelet count and thrombotic complications. Martella et al.[28] and Paterno et al.[29] demonstrated an association between a higher platelet count and thrombosis development; however, in our cohort, it was not statistically significant (p=0.33). Nevertheless, we did not assess platelet function, which is not analyzed by TGA. Several studies have highlighted the relevance of leukemia-related thrombocytopenia and thrombocytopathy in the evaluation of hemostatic complications. However, both conditions appear to be associated with hemorrhagic complications, while an association with thrombotic ones seems unlikely.[7,30–33]

The main strength of this manuscript is that it addresses an important and scarcely explored topic, providing insights into coagulation abnormalities and thrombotic events across different types of acute leukemia. The main limitations of this report are the heterogeneity of the study population, both in terms of disease type and treatment, the small number of patients in each subgroup, the small number of thrombotic events, and the limited follow-up. Hemorrhagic events were not systematically recorded, as the study was designed to focus on thrombotic complications.

**Conclusions.** Our data confirm that thrombin generation is frequently elevated at the onset of acute leukemia. While baseline TGA did not predict thrombotic complications, this finding should not be interpreted as dismissing the clinical utility of TGA. On the contrary, increasing evidence suggests that a dynamic evaluation of TGA parameters throughout key treatment phases (diagnosis, induction, consolidation, remission, and relapse) could be useful in predicting thrombotic and hemorrhagic events in this setting. Moreover, integrating platelet function tests and inflammatory biomarkers could further enhance its predictive and clinical utility in this setting.

**Contributions.** C.A. conceived and designed the study. B.M. wrote the manuscript. M.R. and D.M.S. analyzed the samples. L.A., B.M.L., F.A., and P.A. interpreted the data. B.E. and S.C. critically revised the manuscript. All authors have read and approved the final version of the manuscript and agree to be held responsible for the integrity of the work.

**Data availability.** The data presented in this study are available on request from the corresponding author.

**Ethical statement.** The study was conducted in accordance with the Declaration of Helsinki and its later amendments or comparable ethical standards.

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