Global hemostasis assays in acute myeloid leukemia: results of an observational prospective study

Simona Raso[1,2], Alessandro Lucchesi[3], Mariano Sardo[4], Ombretta Annibali[5], Vincenzo Sucato[6], Marcello Ciaccio[7], Silvana Vitale[7], Alberto Dolce[8], Giulio Giordano[9], Sergio Siragusa[4], Mariasanta Napolitano[4]

[1]*University of Palermo, Department of Surgical, Oncological and Oral Sciences (Di.Chir.On.S.), Palermo, Italy;*
[2]*Department of Hematology and Rare Diseases, V Cervello Hospital, Azienda Ospedaliera Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy;*
[3]*Hematology Unit, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) "Dino Amadori", Meldola, Italy;*
[4]*Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo and Policlinico Paolo Giaccone, Unit of Hematology, Palermo, Italy;*
[5]*Hematology Unit, Campus Bio-medico, University of Rome, Rome, Italy;*
[6]*Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo and Policlinico Cardiology Unit, Paolo Giaccone, Palermo, Italy;*
[7]*Department and U.O.C. Laboratory Medicine, "Paolo Giaccone" University Hospital, Palermo, Italy;*
[8]*Istituto di Statistica, ISTAT, Palermo, Italy;*
[9]*Division of Internal Medicine, Hematology Service, Regional Hospital "A. Cardarelli", Campobasso, Italy*

Arrived: 27 October 2022
Revision accepted: 24 March 2023
Correspondence: Simona Raso
e-mail: simona.raso86@gmail.com

Background - Acute myeloid leukemia (AML) is characterized by a complex spectrum of coagulopathy ranging from hemorrhagic to thrombotic symptoms. To date, platelet count (PLT) and conventional coagulation tests (CCTs) cannot predict hemorrhagic events and thrombotic risk. Thromboelastography (TEG) measures the viscoelastic properties of the clot, thus providing information on the entire process of blood coagulation. The primary aim of the study was to assess the hemostatic balance from AML diagnosis to the end of chemotherapy (CHT) by TEG.

Material and methods - Here we present the results of a prospective study enrolling newly diagnosed AML patients treated with chemotherapy. Patients had complete blood counts (CBCs), TEG and CCTs performed at three time points: 1) diagnosis (T_0); 2) during the first cycle of CHT (T_1); and 3) at the end of CHT (T_2). An algorithm of TEG indirectly calculated thrombin generation (TG). Patients underwent daily follow-up for bleeding and thrombotic episodes up to the time of hospital discharge or death.

Results - Eighty consecutive patients were evaluated; forty were eligible for the study, and 21 completed the entire study. At T_1, maximum amplitude (MA), TG and K-time were significantly shifted toward a hypocoagulability state compared to T_0 ($p<0.05$), while a hypercoagulable state at T_2 was shown by changes in α-angle, MA and TG values. Otherwise, there were no statistically significant differences in CCTs between the evaluated time points.

Discussion - Overall, TEG revealed complex and dynamic coagulation abnormalities in patients with AML according to both the course of disease and therapy. Further studies are needed to investigate more fully the role of TEG in defining the hemostatic profile in patients with AML.

Keywords: *thromboelastography, acute myeloid leukemia, thrombosis, hemorrhage.*

INTRODUCTION

Thrombotic or hemorrhagic complications are frequently reported in hematologic malignancies, with a significant impact on morbidity and mortality[1]. Major and life-threatening bleeding are present in up to 43% and 1% of cases, respectively[2]. Thrombotic complications range from 2 to 12%, according to available studies[3].

The pathogenesis of venous thromboembolism (VTE) in the context of acute myeloid leukemia (AML) is multifactorial and depends on: 1) the procoagulant properties of leukemic cells; 2) patient-related factors; 3) chemotherapy; 4) the presence of a central venous catheter (CVC); and 5) septic complications[4]. The hypercoagulable state affects two classical coagulation pathways. The extrinsic pathway is particularly activated by the release of tissue factor (TF) by blasts and extracellular vesicles (EVs), while the intrinsic pathway is more dependent on mechanisms of adhesion of the blast and activated platelet to the endothelium by molecules such as P-Selectin[5]. It can be assumed that the contribution of the two pathways varies at different stages of the disease and its treatment, as well as in a dependent manner on the heterogeneous biological features that characterize AML.

In contrast, thrombocytopenia can be a common presenting feature of AML; it can be worsened by intensive chemotherapy (CHT), which usually lasts 2-3 weeks, thus increasing the risk of fatal hemorrhages[6,7]. However, since bleeding complications are quite uncommon in non-neoplastic thrombocytopenia, other factors must be involved in order to explain the increased bleeding tendency in AML patients while under treatment[8]. A study comparing platelet reactivity in patients with AML or myelodysplastic syndromes (MDS) and patients with immune thrombocytopenia (ITP) showed that clonal myeloproliferation is associated with changes in platelet function[9]. Interestingly, Bumbea *et al.* recently confirmed altered expression of cytofluorometric markers of platelet activation in some AML patients, suggesting platelet functional defects or denatured signaling[10]. This could be one of the possible explanations for the lack of a close correspondence between platelet count and bleeding events. Platelet aggregation in *in vitro* studies (the outcome of which is unaffected by platelet transfusions) have been shown to predict clinical bleeding within seven days[11].

Conventional coagulation tests (CCTs) are unable to assay interactions between clotting factors, blood cell elements and vascular endothelium[12], and cannot predict and/or guide therapy in acute hemorrhage or predict thrombotic risk in AML. Thromboelastography (TEG) is a global hemostatic test that measures the viscoelastic properties of the clot, thus providing information on the entire process of blood coagulation, from the early phase of clot formation to fibrinolysis[13]. In recent years, TEG has become a valuable tool in detecting coagulopathies and addressing hemostatic and transfusion therapies in different clinical settings, including trauma care, cardiac surgery and liver transplantation[13]. Moreover, it has been adopted to monitor hemorrhages and guide therapy during obstetric surgery within intensive care units (ICUs)[14]. TEG offers several advantages: it is fast and inexpensive, and results can be available within 5-10 minutes (min). TEG has recently been adopted to predict thromboembolic events and hypercoagulability in patients with malignancies, including hematologic malignancies[15-20]. The use of TEG to evaluate global hemostasis may better mirror the *in vivo* coagulation process of patients affected by AML. Finally, the instrument is capable of studying the classic coagulation pathways separately, offering a dynamic assessment of their respective balance. Therefore, a prospective observational pilot study was performed with the primary aim of assessing the hemostatic balance in patients with AML at diagnosis and during different phases of CHT with TEG. The secondary aim of this study was to specifically analyze TEG profiles in patients with clinically relevant thrombotic and/or hemorrhagic complications.

MATERIALS AND METHODS

Study population

Eligible patients were ≥18 years of age with a newly confirmed diagnosis of AML. Exclusion criteria were: diagnosis of acute promyelocytic leukemia (APL), known coagulation disorders, known liver diseases, concomitant solid neoplasms, and regular use of anticoagulants. The study was approved by the Ethics Committee of University Hospital of Palermo (protocol No. 04/2018). All enrolled subjects provided informed consent according to the principles of the Declaration of Helsinki. All enrolled subjects received treatment regimens in accordance with international guidelines[21].

Laboratory evaluation

Assessment of complete blood counts (CBCs) and CCTs, including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FBG), and TEG, were performed at the following time points: 1) diagnosis of AML (T_0); 2) seven days after the start of CHT (T_1); and 3) at recovery (independently of remission or resistance)

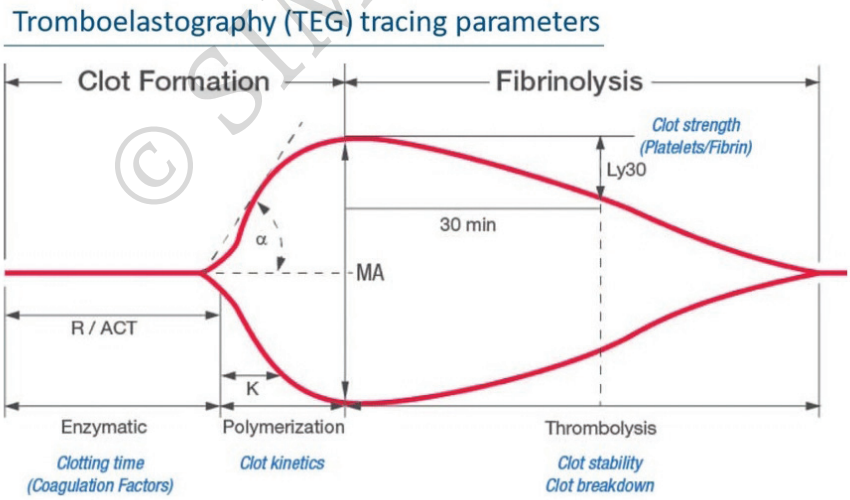
from the first cycle of CHT (T_2). Baseline characteristics (age, comorbidities, and treatment received) were recorded in a dedicated database accessible only to study staff and protected by a password. Blood samples were collected using 19-21-gauge needles and 22-23-gauge needles for adults with good or difficult venous access, respectively, or from a central venous catheter (the first 5 mL was discarded) and analyzed within 2 hours (h) after collection. Coagulation samples were collected in citrated blood (anticoagulated with 10^9 mmol/L (3.2%) trisodium citrate at least 24 h after a platelet or red blood cell (RBC) transfusion in the absence of any concomitant anticoagulant treatment. Patients were followed daily for bleeding and thrombotic episodes until discharge or death. Bleeding was graded from 1 to 4 according to the WHO criteria. Thrombosis included venous thromboembolism (VTE), superficial venous thrombosis (SVT), and arterial thrombosis, which were first clinically suspected and then objectively diagnosed and confirmed using standard diagnostic testing (i.e., ultrasound and computed tomography).

Thromboelastography

TEG was performed on citrated whole blood samples within 2 h after blood collection by a Haemoscope Thrombolastograph® Haemostasis Analyser Model 5000, software V.4. (Haemoscope Corporation, Niles, IL, USA). Testing was carried out by the same trained biomedical scientists using citrated blood samples (340 µL that were recalcified with 20 µL of 0.2 M $CaCl^2$. Reaction time (R, min), alpha-angle (a-angle, °), kinetic time (K, min) and maximum amplitude (MA, mm) of the TEG parameters were recorded (**Figure 1**). The normal reference ranges, defined by internal control, were considered: R 9-27 min, K 2-9 min, a 22°-58°, and MA 44-64 mm. Internal controls were obtained from 40 healthy individuals with a mean age of 60.57±16.45 years; 13 males (mean 62, range 46±19.68) and 17 females (mean range 59.12±13.95). An algorithm of the instrument indirectly calculated thrombin generation (TG) from the first derivative of the TEG waveform[22] and 494-824 mm/min was considered normal range. Quality control was maintained per the manufacturer's instructions.

Blood count and coagulation tests

Complete blood count was performed using a COBAS INTEGRA 800 biochemical analyzer (Roche, Basel, Switzerland). A-PTTs and PTs were performed using the automated coagulometer ACL TOP 750 series (Instrumental Laboratory, Werfen, Barcelona, Spain), and



Components	α Alpha angle of tangent line	K Clot formation time (CFT)	LY_{30} Lysis Index	R Reaction time	MA Maximum Amplitude
Definition	Angle	Time from 2mm-20mm amplitude	Percent loss of amplitude at 30min after MA	Time till initiaton of fibrin clot formation	Amplitude measure at clot strength

Figure 1 – Thromboelastography (TEG) parameters

FBG was assayed by the Clauss method using reagents and methodology according to the manufacturer's instructions. Platelet-poor plasma (PPP) was obtained by a 2-step centrifugation procedure: centrifugation of citrated blood at 10,000 × *g* for 5 min and then at 1,500 × *g* for 3 min[23]. All samples were collected at the appropriate time point, and CBC and FBG measurements were performed within 2 h after blood collection. All the other tests were performed batchwise in stored PPP (–80°C), which was frozen in aliquots within 2 h after blood collection. Normal reference ranges were taken from controls: PT-ratio 0.8-1.2, A-PTT ratio 0.8-1.2, FBG 150-450 mg/dL.

Statistics

Median and range were used to represent descriptive statistics, and the position parameter of the main variable distributions and repeated measures analysis of variances (Manova) was used since the same parameter was measured under different conditions on the same subjects. The equality of variances of the differences between measurements, which is an assumption of ANOVA with a repeated measures factor, was tested with the Huynh and Feldt sphericity test. Additionally, we performed the Test of Within Subjects Effects to verify whether there was a significant difference between the different measurements. Then, we performed *post hoc* pairwise comparisons where the different measurements were compared to each other. Bonferroni correction for multiple comparisons was applied for p values. $p<0.05$ was considered statistically significant. The sample size was not calculated using a formal sample size calculation method, but rather the entire population of patients in the hospital was treated as a sample. We have thus investigated the entire specific and eligible population of patients in our center, as it was considered a representative sample of the target population, sufficient to achieve the research goals, and to increase the representativeness of the sample and generalizability of the findings. Statistical analyses and graphs were performed with MedCalc version 19.8, (MedCalc Software Ltd, Ostend, Belgium).

RESULTS

Patients

From May 2018 to December 2019, 80 consecutive patients affected by AML were admitted to the Unit of Hematology of the University Hospital of Palermo. Of these, 18 patients moved to another hospital, 12 patients were not treated with CHT (receiving exclusively supportive therapy at home or continuative therapy with hydroxyurea), and eight patients died from infective complications after diagnosis and before the beginning of CHT; two patients were affected by APL.

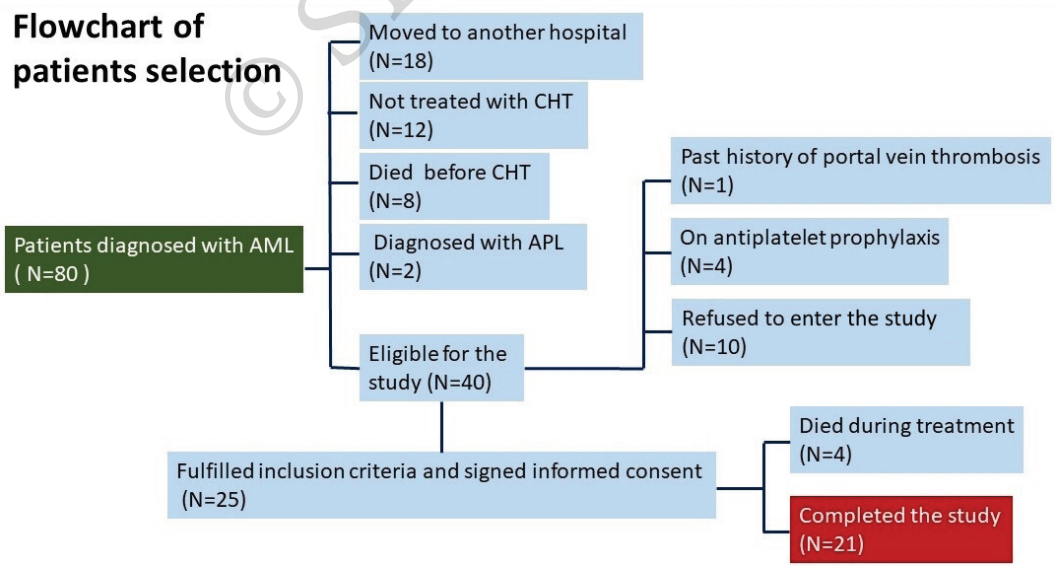


Figure 2 – Flowchart of patients' selection
AML: acute myeloid leukemia; CHT: chemotherapy; APL: acute promyelocytic leukemia

The remaining 40 patients were thus potentially eligible for the study; however, 15 subjects were not enrolled in the study because ten patients refused the study, one patient had a past history of portal vein thrombosis, and four patients were on antiplatelet prophylaxis at diagnosis. Thus, 25 patients fulfilled the inclusion criteria and agreed to be enrolled in the study. However, four patients did not reach the end of CHT (T_2) due to death during treatment. In conclusion, at the end of the study, 21 patients had completed the three planned time points (T_0, T_1, T_2), and data related to these subjects were analyzed (**Figure 2**). Regarding disease molecular characteristics, we detected AML-NOS in 18 patients. *NPM1* mutation and AML with myelodysplasia-related changes were found in two patients and one patient, respectively. The median time of follow-up was 36 days (range 18-69).

According to standard practice, when the platelet count (PLT) was <10×10^9/L, patients received a prophylactic transfusion with one batch of platelet (PLT) concentrate, while the target hemoglobin level (Hb) for transfusion was 8.0 mg/dL and 8.5 mg/dL in selected cases (active infection such as acute pneumonia and known cardiovascular comorbidities)[24]. Patients received oral antifibrinolytic prophylaxis when PLT was <30×10^9/L. **Table I** shows the clinical and demographic characteristics of the patients. **Table II** and **Table III** summarize CBC and CCT results, and TEG parameters at each time point, respectively.

Table I - *Patients' demographics and clinical characteristics*

No. patients	21
Age, years, median (range)	68 (32-86)
Sex F/M, No. (%)	9 (42.8)-12 (57.2)
Time of follow-up: days, median (range)	36 (18-69)
Cardiovascular risk factors: No. (%)	
DM	6 (28)
Hypertension	18 (85)
Obesity	3 (14)
COPD	8 (38)
Hypercholesterolemia	2 (9)
No comorbidity	2 (9)
Treatment plan: No. (%)	
Cytarabine+daunorubicine (7+3)	13 (52)
FLAG	1 (4)
Azacytidine	4 (19)
Decitabina	3 (14)

AML: acute myeloid leukemia; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; FLAG: fludarabine, cytarabine, and granulocyte colony-stimulating factor.

Hemostasis results at diagnosis

TEG parameters at baseline (T_0) showed the highest proportion of values outside the interval reference range; R and MA were reduced in 47% and 35% of patients, respectively; TG and K were also reduced (25% and 27% of results, respectively, below reference intervals), while the α-angle exceeded the normal range level in 14% of results. The median PLT was 36×10^9/L (range 4.0-240×10^9/L). Regarding CCTs, the mean values of APTT and the PT ratio were within the normal reference range, and FBG was above the normal range in 11 patients.

Table II - *Laboratory and conventional coagulation tests*

Tests	T_0	T_1	T_2	Pairwise comparison
	Median (range)	Median (range)	Median (range)	Time and p value (if significant)
Hb (N.V. 12-18 mg/dL)	8.8 (7.6-14.0)	8.7 (7.5-9.5)	9.0 (8.1-12.0)	T_2/T_1-0.0015
Hct (N.V. 37-52%)	26.7 (22-40.3)	25.9 (22-35.9)	28.0 (23.0-36.3)	None
WBC (N.V. 4-11×10^9/L)	22.2 (0.420-239)	1.53 (0.170-44.52)	3.7 (0.90-10)	T_1/T_0-0.054 T_2/T_0-0.035
Neu (N.V. 2-8×10^9/L)	1.31 (0.110-53)	0.410 (0.10-7.84)	1.690 (240-6.02)	None
Lym (N.V. 1-5×10^9/L)	3.69 (0.207-125)	0.610 (0.120-9.28)	0.850 (0.470-4.14)	None
Mono (N.V. 0.16-1.0×/L)	11.75 (0.010-91)	0.240 (0-34.55)	440 (0.03-2.14)	T_2/T_0-0.036
PLT (N.V. 150-450×10^9/L)	36 (4-240)	34 (2-212)	149 (8-387)	T_2/T_1-0.004
PT-ratio (N.V. 0.8-1.2)	1.18 (0.86-1.95)	1.05 (0.9-1.39)	1.11 (0.87-1.39)	None
APTT-ratio (N.V. 0.8-1.2)	0.7 (0.6-2.1)	0.9 (0.4-1.4)	1.0 (0.9-2.7)	None
FBG (N.V. 150-450 mg/dL)	429 (39-1.107)	305 (108-771)	390 (103-1.107)	None
Blast cells count (%)	70 (55-90)	-	4 (1-85)	-

N.V.: normal value

Hemostasis results during chemotherapy

At T_1, almost all TEG parameters (**Table III**) shifted toward a hypocoagulable state when compared to T_0: MA (median, range: 56, 25.9-80 *vs* 50, 8.6-66.7 mm; p=0.0048), α-angle (median, range: 58.7°, 10.7°-77.2° *vs* 50°, 11.6°-76.7°; p=0.035) and TG (median, range: 672, 310.9-959.6 *vs* 621, 164.7-864 mm/min; p=0.0175) were decreased, while K was increased (median, range: 2.0, 0.3-10.5 *vs* 4.7, 0.8-19 min; p=0.018). Otherwise, R was shorter at T_0 (p>0.05). We found no significant differences in CCTs compared to T_0 and no correlation with the global hemostasis tests. Finally, at T_1, the white blood cell count (WBC) was lower than that at T_0 (median, range: 22.2×10⁹/L, 0.420-239×10⁹/L *vs* 1.53×10⁹/L, 0.170-44.52×10⁹/L; p=0.054), while PLT was only slightly reduced (median, range: 34, 2-212×10⁹/L).

Hemostasis results after chemotherapy

At T_2, the α-angle and MA were significantly wider than at T_1 (median, range, alpha-angle 64°, 37.4°-74.7° *vs* 50°,11.6°-76.7°; p=0.0017; MA 66, 28.1-77 *vs* 50, 8.6-66 mm; p=0.0001), and TG increased (median, range: 804, 313-995 *vs* 621, 164.7-864 mm/min; p=0.0008). K was significantly shorter (median, range: 2.5, 0.9-8.2 *vs* 4.7, 0.8-19 min; p=0.0061). R was not significantly reduced compared to the other time points. PLT (p=0.004) and Hb (p=0.0015) were markedly increased compared to T_1, while WBC was reduced in comparison with values at diagnosis (p=0.035). Analysis of CCTs showed no differences in comparison to the other time points (**Table II**).

Bleeding and thrombotic complications within the course of AML

Among 21 patients, 14 (66%) had hemorrhagic symptoms: three had WHO grade 4 bleeding, including central nervous system (CNS) hemorrhage (No.=1), bleeding secondary to ruptured spleen (No.=1) and one subretinal hemorrhage; two experienced WHO grade 3 epistaxis; and nine had WHO grade 2 bleeding episodes, including hematuria (No.=2), skin petechiae (No.=4), ecchymosis (No.=2), and gum bleeding (No.=1). In ten patients, symptoms were reported during CHT (T_1) (2 on hypomethylating agents and 8 on a myeloablative regimen), while symptoms were reported in four patients at diagnosis (T_0). Overall, three patients (14%) experienced thrombotic complications: one deep vein thrombosis (DVT), one CVC-related thrombosis, and one portal vein thrombosis (PVT). In detail, PVT was diagnosed before CHT administration (T_0), while the other two thrombotic complications occurred during CHT (T_1) (**Table IV**). There were no significant differences in terms of conventional and global coagulation test results between patients experiencing bleeding and thrombotic complications and those without complications at T_0, T_1, or T_2. When CCTs and TEG results were compared according to the type of complications (thrombosis or hemorrhage), no differences were found at any of the time points analyzed.

In detail, TEG variables showed no significant differences between the groups with and without hemostatic complications, but this result could depend on the sample size of our study. The median value of R and K for the group with VTE (3 subjects) was 8.7 min and 2.7 min, respectively; this was not significantly different from the median values of the group without thrombotic complications (R 7.6 min and K 1.85 min, p=0.16 and p=0.85, respectively). Interestingly, in 2 out of 3 cases of VTE, TEG at T_0 showed decreased K and R values with respect to reference values. However, all the enrolled patients experiencing clinically

Table III - *Thromboelastography parameters during all time points of the study*

Parameters	T_0	T_1	T_2	Pairwise comparison
	Median (range)	Median (range)	Median (range)	Time and p value (if significant)
R (N.V. 9-27 min)	8.9 (5.2-16.8)	8.1 (5.8-14.0)	8.0 (6.1-11.2)	None
K (N.V. 2-9 min)	2.0 (0.3-10.5)	4.7 (0.8-19.0)	2.5 (0.9-8.2)	T_1/T_0-0.0181 T_2/T_1-0.0061
MA (N.V. 44-64 mm)	56.0 (25.9-80.0)	50.0 (8.6-66.7)	66 (28.1-77.0)	T_1/T_0-0.0048 T_2/T_1-0.0001
a-angle (N.V. 22-58°)	58.7 (10.7-77.2)	50.0 (11.6-76.7)	64.0 (37.4-74.7)	T_1/T_0-0.035 T_2/T_1-0.0017
TG (N.V. 494-824 mm/min)	672 (310.9-959.6)	621 (164.7-864)	804 (313-995.0)	T_1/T_0-0.0175 T_2/T_1-0.0008

R: reaction time; K: kinetic time; MA: maximum amplitude; a-angle: alpha-angle; TG: thrombin generation; N.V.: normal value.

Table IV - *Thrombotic or bleeding complications*

Bleeding complications (No.=14)	T_0	WHO	T_1	WHO	T_2	WHO
Central nervous system (No.=1)	--	--	1	4	--	--
Ruptured spleen (No.=1)	--	--	1	4	--	--
Subretinal hemorrhage (No.=1)	--	--	1	4	--	--
Gum bleeding (No.=1)	1	2	--	--	--	--
Ecchymosis (No.=2)	1	2	1	2	--	--
Skin petechiae (No.=4)	2	2	2	2	--	--
Hematuria (No.=2)	0	--	2	3	--	--
Epistasis (No.=2)	0	--	2	3	--	--
Thrombotic complications (No.=3)	**T_0**	**WHO**	**T_1**	**WHO**	**T_2**	**WHO**
Deep vein thrombosis (DVT, No.=1)	-	-	1	-	-	-
Portal vein thrombosis (No.=1)	1	-	-	-	-	-
CVC-related thrombosis (No.=1)	--	-	1	--	--	--

--: No thrombotic or bleeding episodes occurred; CVC: central venous catheter.

bleeding showed a trend toward a hypocoaguable state on TEG at T_0, with longer R (median 9.7 *vs* 8.1 min; p=0.56) and K (median 3.1 *vs* 2.3 min; p= 0.65) compared to their non-bleeding counterparts.

DISCUSSION

Our results show that TEG is able to identify changes in the hemostatic profile of patients with AML according to the course of the disease. A large dispersion of TEG parameters was observed in newly diagnosed patients with AML, showing a wide variability from hypocoagulability to hypercoagulability. In approximately half of patients, the most frequent sign of hypercoagulability was a shorter R, as a likely expression of increased involvement of plasma factors in the hemostatic process. Indeed, like PT-ratio and APTT, R can be considered a surrogate marker of clotting factor levels[13]. However, in the analyzed cohort of patients, the PT ratio and APTT were not abnormal at diagnosis. Unfortunately, we have not assayed coagulation factors; however, some authors have previously reported an increase in coagulation factor plasma levels in patients with acute leukemia before CHT[25]. According to these findings, TEG at diagnosis confirms the coagulation activation frequently observed in neoplastic patients, and underlines the heterogeneity and complexity of hemostatic alterations in patients with AML.

Comparing TEG results between all time points of the study, we found that the most clinically and statistically significant changes emerged during and after CHT. In detail, TEG identifies a hypocoagulable state during CHT characterized by a slower rate of clot formation time (K), weaker clots (MA), and a marked reduction in TG levels with respect to the time of diagnosis. Kim *et al.* showed that, in patients with hematologic malignancies, significant determinants of the K value were coagulation factor VII, PLT and FBG[26]. In our cohort, FBG levels were within the normal range, and patients presented mild thrombocytopenia. Considering that a reduction in the level of FVII in response to CHT has been reported in hematologic patients[27], we may suppose a reduction in FVII to be a possible cause of hypocoagulability. The presence of a shorter R could be in contrast with our hypothesis. However, first, this result was not statistically significant; second, it may reflect the concomitant activation of endothelial cells and CHT-induced upregulation of tissue factor levels[28]. Our analysis provides results consistent with another study on t-PA ROTEM in chemotherapy-induced thrombocytopenia (CIT) patients showing a decreased K and lower MA[29]. Previous studies have shown that platelets influence fibrin formation and clot structure[30], suggesting that the weakness of clots (as measured by MA) might be due to the low PLT. Considering that PLT inadequately predicts bleeding in non-malignant thrombocytopenia, it cannot be considered the only factor responsible for bleeding risk in CIT. Thus, altered platelet function during CHT could help explain the changes seen in TEG. A recent study indicated impaired platelet function, without compensation by higher coagulation

activity, as possibly responsible for the low hemostatic profile observed in thrombocytopenic patients undergoing cytoreductive treatment[27].

At the same time, a concomitant absence of compensatory higher coagulant activity during CHT is suggested by slower clot formation and reduced TG levels. In general, it has been reported that standard TEG is unlikely to provide a comprehensive or sensitive reflection of impaired platelet function[31]; however, Bao *et al.*[32] showed, in 226 patients with leukemia, that the combination of PLT and MA had a synergistic effect on the prediction of bleeding risk, suggesting that TEG parameters could reflect not only platelet number but also platelet function. Thromboelastograph® Platelet Mapping™ (Haemoscope Corporation, Niles, IL, USA) is a modified TEG that can also measure platelet function. The potential of this methodology is related to assessing all functional roles of platelets in hemostasis: thrombin generation, clot formation, clot retraction, and lysis[33]. In patients with AML, where platelet count and function seem to play a fundamental role, the employment of this instrument could be useful and more comprehensive than standard TEG. Taken together, our results suggest that, during CHT, patients with AML may develop a state of hypocoagulability that cannot be simply attributed to the degree of thrombocytopenia but could involve coagulant activity and platelet function. Regarding the significant hypercoagulable changes underlined in almost all TEG variables after CHT, there are several aspects to consider. On the one hand, the results of TEG could indicate the restoration of the coagulation balance and physiology after a hypocoagulative state induced by CHT. On the other hand, the alterations may indicate a residual hypercoagulable effect of CHT, as previously shown in other hematologic neoplasms, including APL[28]. This last hypothesis is supported by the observation that approximately 5% of adult patients with AML may experience VTE within the first two months after diagnosis[34]. However, in the current analysis, most cases of VTE occurred at T_1, when TEG showed a state of hypocoagulability. A hypothesis explaining these events is that at the time of TEG analysis, pro-thrombotic variations responsible for VTE were resolved, giving space to pro-hemorrhagic abnormalities that are predominant in these patients. It should be noted that in all episodes of VTE, patients showed infectious complications at diagnosis, which may have exerted a transient prothrombotic action. TEG variables showed no significant differences between the groups with and without hemostatic complications, but this could depend on the sample size of our study. Moreover, TEG performed the day before the onset of symptomatology revealed signs of hypocoagulability in the patient with CNS hemorrhage and concomitant PLT >10x10^9/L. This is in line with other studies, showing that TEG parameters are better at discriminating between patients having significant bleeding alone or in association with the PLT than with the PLT alone[32]. Prophylaxis and therapy of hemostatic complications is still a challenging issue in patients with AML[35,36], and it is important to find tools to predict which patients are at highest thrombotic or bleeding risk and might benefit most from therapeutic interventions.

Our data suggest that TEG may detect changes in addition to thrombocytopenia in the hemostatic status of patients with AML early during chemotherapy. The potential application of TEG as a global test, perhaps in combination with platelet function tests, should be investigated to monitor coagulation and to guide hemostatic and antithrombotic treatment in AML. TEG is fast; results can be available within 5-10 min, which could be an advantage in the management of severe acute hemorrhages. Moreover, it is easy to handle, and often available in intensive care and cardiac surgery units, so it could be regularly used not only in specialized coagulation centers but also in peripheral hospitals.

This study had several limitations. First, we studied patients after receiving distinct treatment regimens, but the number of patients was too small to perform a stratification based on the type of CHT. Second, we estimated TG levels by a TEG algorithm and not by the calibrated automated thrombogram (CAT) method[37]. This last technique involves a time-consuming and complex process, which prevents its use for rapid diagnosis; thus, it was not in line with our study objectives. Furthermore, many of the suspicions regarding the hemorrhagic tendency of AML patients at diagnosis fall on platelet function, so it would be appropriate to integrate Platelet Mapping™ technology into future studies. Finally, the small sample size limited the statistical significance. However, we would like to highlight that, to our knowledge,

this is the first study to systematically evaluate global hemostasis tests and coagulation parameters in patients with AML during different phases of disease, including after treatment.

CONCLUSIONS

In conclusion, our findings show that TEG alone, even if able to detect dynamic coagulation abnormalities in AML patients during the disease course and treatment, failed to overcome the limits of standard coagulation tests. Further studies are needed to investigate the role of TEG as a tool to define the hemostatic profile and offer individualized transfusion support and antithrombotic strategies in patients with AML.

ACKNOWLEDGMENTS

Authors are grateful to Professor Giuseppe Avvisati for revising the manuscript and Elsevier editing system for the language revision of this paper. Authors would like to thank Lucio Lo Coco for its support in the development of this research.

AUTHORSHIP CONTRIBUTIONS

SR: analysis of data, writing, original draft preparation; VS and MS: data collection; AL, GG and OA: data curation; AD: statistical analysis; SV and MM laboratory analysis; SS: review and editing; MN: conceptualization, supervision and review. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

MN acted as consultant for Bayer, BIOFVIIIx, Novonordisk, Amgen, and received speaker fees from: Kedrion, Octapharma, Baxalta, CSL Behring, Novonordisk, Bayer, Sobi.Takeda. SS acted as consultant for Bayer, Novonordisk, Amgen, Biomarin, Novartis and received speaker fees from: Baxalta, CSL Behring, Novonordisk, Bayer, Sobi.Takeda, BioFVIIIx All other Authors have no relevant conflicts of interest to declare.

REFERENCES

1. Falanga A, Rickles FR. Management of thrombohemorrhagic syndromes (THS) in hematologic malignancies. Hematology Am Soc Hematol Educ Program 2007; 165-171. doi: 10.1182/asheducation-2007.1.165.
2. Rickles FR, Falanga A, Montesinos P, Sanz MA, Brenner B, Barbui T. Bleeding and thrombosis in acute leukemia: what does the future of therapy look like? Thromb Res. 2007; 120 (Suppl 2): S99-S106. doi: 10.1016/S0049-3848(07)70137-8.
3. Mohren M, Markmann I, Jentsch-Ullrich K, Koenigsmann M, Lutze G, Franke A. Increased risk of venous thromboembolism in patients with acute leukaemia. Br J Cancer 2006; 94: 200-202. doi: 10.1038/sj.bjc.6602945.
4. Annibali O, Napolitano M, Avvisati G, Siragusa S. Incidence of venous thromboembolism and use of anticoagulation in hematological malignancies: critical review of the literature. Crit Rev Oncol Hematol 2018; 124: 41-50. doi: 10.1016/j.critrevonc.2018.02.003.
5. Del Principe MI, Del Principe D, Venditti A. Thrombosis in adult patients with acute leukemia. Curr Opin Oncol 2017; 29: 448-454. doi: 10.1097/CCO.0000000000000402.
6. Damon LE, Rugo HS, Ries CA, Linker CA. Post-remission cytopenias following intense induction chemotherapy for acute myeloid leukemia. Leukemia 1994; 8: 535-541. PMID: 8152248.
7. Chen CY, Tai CH, Cheng A, Wu HC, Tsay W, Liu JH, et al. Intracranial hemorrhage in adult patients with hematological malignancies. BMC Med 2012; 10: 97. doi: 10.1186/1741-7015-10-97.
8. Stanworth SJ, Estcourt LJ, Powter G, Kahan BC, Dyer C, Choo L, et al. A no-prophylaxis platelet-transfusion strategy for hematologic cancers. N Engl J Med 2013; 368: 1771-1780. doi: 10.1056/NEJMoa1212772.
9. Psaila B, Bussel JB, Frelinger AL, Babula B, Linden MD, Li Y, et al. Differences in platelet function in patients with acute myeloid leukemia and myelodysplasia compared to equally thrombocytopenic patients with immune thrombocytopenia. J Thromb Haemost 2011; 9: 2302-2310. doi: 10.1111/j.1538-7836.2011.04506.x.
10. Bumbea H, Vladareanu AM, Dumitru I, Popov VM, Ciufu C, Nicolescu A, et al. Platelet defects in acute myeloid leukemia-potential for hemorrhagic events. J Clin Med 2021; 11: 118. doi: 10.3390/jcm11010118.
11. Just Vinholt P, Højrup Knudsen G, Sperling S, Frederiksen H, Nielsen C. Platelet function tests predict bleeding in patients with acute myeloid leukemia and thrombocytopenia. Am J Hematol. 2019; 94: 891-901. doi: 10.1002/ajh.25512.
12. Friedmann AM, Sengul H, Lehmann H, Schwartz C, Goodman S. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A reevaluation of prophylactic platelet transfusions. Transfus Med Rev 2002; 16: 34-45. doi: 10.1053/tmrv.2002.29403.
13. Whiting D, DiNardo JA. TEG and ROTEM: technology and clinical applications. Am J Hematol 2014; 89: 228-232. doi: 10.1002/ajh.23599.
14. Amgalan A, Allen T, Othman M, Ahmadzia HK. Systematic review of viscoelastic testing (TEG/ROTEM) in obstetrics and recommendations from the women's SSC of the ISTH. J Thromb Haemost 2020; 18: 1813-1838. doi: 10.1111/jth.14882.
15. Toukh M, Siemens DR, Black A, et al. Thromboelastography identifies hypercoagulablilty and predicts thromboembolic complications in patients with prostate cancer. Thromb Res. 2014; 133(1): 88-95. doi: 10.1016/j.thromres.2013.10.007.
16. Treliński J, Misiewicz M, Robak M, Smolewski P, Chojnowski K. Assessment of rotation thromboelastometry (ROTEM) parameters in patients with multiple myeloma at diagnosis. Thromb Res 2014; 133: 667-670. doi: 10.1016/j.thromres.2014.01.011.
17. Giordano P, Molinari AC, Del Vecchio GC, Saracco P, Russo G, Altomare M, et al. Prospective study of hemostatic alterations in children with acute lymphoblastic leukemia. Am J Hematol 2010; 85: 325-330. doi: 10.1002/ajh.21665.
18. Lim HY, O'Malley C, Donnan G, Nandurkar H, Ho P. A review of global coagulation assays - Is there a role in thrombosis risk prediction? Thromb Res 2019; 179: 45-55. doi: 10.1016/j.thromres.2019.04.033.
19. Ko RH, Sposto R, Goodarzian F, Carmona R, Vasquez S, Young G. Thromboelastography to detect hypercoagulability in patients with newly diagnosed acute lymphoblastic leukemia. Blood 2015; 126: 1118. doi: 10.1182/blood.V126.23.1118.1118.
20. Walsh M, Kwaan H, McCauley R, Marsee M, Speybroeck J, Thomas S, et al. Viscoelastic testing in oncology patients (including for the diagnosis of fibrinolysis): review of existing evidence, technology comparison, and clinical utility. Transfusion 2020; 60 (Suppl 6): S86-S100. doi: 10.1111/trf.16102.
21. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424-447. doi: 10.1182/blood-2016-08-733196.

22. Rivard GE, Brummel-Ziedins KE, Mann KG, Fan L, Hofer A, Cohen E. Evaluation of the profile of thrombin generation during the process of whole blood clotting as assessed by thrombelastography. J Thromb Haemost 2005; 3: 2039-43. doi: 10.1111/j.1538-7836.2005.01513.x.
23. Kitchen S, Olson JD, Preston FE. *Quality in laboratory hemostasis and thrombosis*. London: Wiley-Blackwell, 2009. 215 p. doi: 10.1002/9781444303575.
24. Slichter SJ, Kaufman RM, Assmann SF, McCullough J, Triulzi DJ, Strauss RG, et al. Dose of prophylactic platelet transfusions and prevention of hemorrhage. N Engl J Med 2010; 362: 600-613. doi: 10.1056/NEJMoa0904084.
25. Burley K, Salem J, Phillips T, Reilly-Stitt C, Marks DI, Tunstall O, et al. Evaluation of coagulopathy before and during induction chemotherapy for acute lymphoblastic leukaemia, including assessment of global clotting tests. Blood Cancer J 2017; 7: e574. doi: 10.1038/bcj.2017.54.
26. Kim SY, Gu JY, Yoo HJ, Kim JE, Jang S, Choe S, et al. Benefits of thromboelastography and thrombin generation assay for bleeding prediction in patients with thrombocytopenia or hematologic malignancies. Ann Lab Med 2017; 37: 484-493. doi: 10.3343/alm.2017.37.6.484.
27. Baaten CCFMJ, Moenen FCJI, Henskens YMC, Swieringa F, Wetzels RJH, van Oerle R, et al. Impaired mitochondrial activity explains platelet dysfunction in thrombocytopenic cancer patients undergoing chemotherapy. Haematologica 2018; 103: 1557-1567. doi: 10.3324/haematol.2017.185165.
28. Walsh M, Moore EE, Moore H, Thomas S, Lune SV, Zimmer D, et al. Use of viscoelastography in malignancy-associated coagulopathy and thrombosis: a review. Semin Thromb Hemost 2019; 45: 354-372. doi: 10.1055/s-0039-1688497.
29. Heubel-Moenen FCJI, Henskens YMC, Verhezen PWM, Wetzels RJH, Schouten HC, Beckers EAM. Fibrinolysis in patients with chemotherapy-induced thrombocytopenia and the effect of platelet transfusion. J Thromb Haemost 201; 17: 1073-1084. doi: 10.1111/jth.14465.
30. Mutch NJ, Engel R, Uitte de Willige S, Philippou H, Ariëns RA. Polyphosphate modifies the fibrin network and down-regulates fibrinolysis by attenuating binding of tPA and plasminogen to fibrin. Blood 2010; 115: 3980-3988. doi: 10.1182/blood-2009-11-254029.
31. Bowbrick VA, Mikhailidis DP, Stansby G. Value of thromboelastography in the assessment of platelet function. Clin Appl Thromb Hemost. 2003; 9(2): 137-142. doi: 10.1177/107602960300900208.
32. Bao HX, Du J, Chen BY, Wang Y. The role of thromboelastography in predicting hemorrhage risk in patients with leukemia. Medicine (Baltimore) 2018; 97: e0137. doi: 10.1097/MD.0000000000010137.
33. Weitzel NS, Weitzel LB, Epperson LE, Karimpour-Ford A, Tran ZV, Seres T. Platelet mapping as part of modified thromboelastography (TEG®) in patients undergoing cardiac surgery and cardiopulmonary bypass. Anaesthesia 2012; 67: 1158-1165. doi: 10.1111/j.1365-2044.2012.07231.x.
34. De Stefano V, Sorà F, Rossi E, Chiusolo P, Laurenti L, Fianchi L, et al. The risk of thrombosis in patients with acute leukemia: occurrence of thrombosis at diagnosis and during treatment. J Thromb Haemost 2005; 3: 1985-1992. doi: 10.1111/j.1538-7836.2005.01467.x.
35. Napolitano M, Saccullo G, Marietta M, Carpenedo M, Castaman G, Cerchiara E, et al. Platelet cut-off for anticoagulant therapy in thrombocytopenic patients with blood cancer and venous thromboembolism: an expert consensus. Blood Transfus 2019; 17: 171-180. doi: 10.2450/2018.0143-18.
36. Napolitano M, Valore L, Malato A, Saccullo G, Vetro C, Mitra ME, et al. Management of venous thromboembolism in patients with acute leukemia at high bleeding risk: a multi-center study. Leuk Lymphoma 2016; 57: 116-119. doi: 10.3109/10428194.2015.1046864.
37. Tripodi A. Thrombin generation assay and its application in the Clinical Laboratory. Clin Chem 2016; 62: 699-707. doi: 10.1373/clinchem.2015.248625.