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Diagnosis and management of coagulation derangements in patients with acute leukemia: is there a potential role for thromboelastography?

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1. Abstract

The primary aim of this study was to assess with thromboelastography the coagulation balance in patients affected by acute leukemia, from diagnosis to the end of first cycle of chemotherapy.

2. Summary

Background: Acute leukemia (AL) is characterized by a complex spectrum of coagulopathy ranging from a high bleeding risk to thrombotic risk, varying according to disease phases and treatments. To date platelet count and conventional coagulation tests (CCTs) have been unable to predict thrombotic and hemorrhagic risk in AL.

Objectives: Thromboelastography (TEG) is a global haemostatic test that measures the viscoelastic properties of the clot, thus providing information on the entire process of blood coagulation. The primary aim of this study was to assess with TEG the coagulation balance in patients with AL, from diagnosis to the end of first cycle of chemotherapy (CHT).

Methods: Assessment of CCTs and TEG were made at the following time points: 1) Diagnosis of AL (T0); 2) during the first cycle of CHT (T1); 3) during myelosuppression T2 (PLT $\leq$ $30x10^9$/L, absolute neutrophil count < 1000/mmc); 4) At the end of the first cycle of CHT (T3). Patients were followed-up for bleeding and thrombotic episodes daily up to the time of hospital discharge or death.

Results: Forty consecutive patients were included. Comparing TEG results, we found that the most clinically significant changes emerged during CHT, including the myelosuppression phase, and after CHT. TEG identifies a hypocoagulable state on CHT, exacerbated during myelosuppression, and a hypercoagulable state after CHT. When CCTs and TEG results were compared based on the type of complications (thrombosis or hemorrhages), no differences were found at each analyzed time point.

Conclusions: Cumulative, our findings showed the capacity of TEG revealing complex and dynamic abnormalities in patients with AL according to course of disease and treatment, respect coagulation test. Further studies will investigate the role of TEG in defining hemostatic profile and in individualizing the approach to transfusion and anticoagulant therapy/prophylaxis in patients with AL.

3. Background

Hemostatic balance alterations in either thrombotic or haemorrhagic sense are frequently reported in haematological neoplasms with a significant impact on morbidity and mortality.[1,2] Hematological patients treated with chemotherapy, experience clinically significant bleeding in up to 43% of cases and life-threatening bleeding in 1%. [3] Patients affected by acute leukemia experience specific abnormalities of the hemostatic system. Intensive chemotherapy is the backbone of first line treatment of acute leukemia, it causes bone marrow failure and severe thrombocytopenia usually lasting for 2–3 weeks.[4]. Severe thrombocytopenia predisposes patients to an increased risk of fatal hemorrhages. Despite the high bleeding risk, the incidence of venous thromboembolism (VTE) in acute leukemia is also not negligible ranging from about 2% up to 12% according available studies [2]. Approximately 5% of adult patients with acute leukemia may experience VTE within the first 2 months after diagnosis [5]. The pathogenesis of VTE in the context of acute leukemia is multifactorial depending on: 1) pro-coagulant properties of leukemic cells; 2) patients related factors; 3) chemotherapy; 4) presence of central venous catheter (CVC) and of septic complications. [6] In summary acute leukemia is characterized by a complex spectrum of coagulopathy ranging from a high bleeding risk to thrombotic risk, varying according to disease phases and treatments.[7] Conventional coagulation tests (CCTs) include prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen (FBG) concentration and D-dimer (DD) level. These tests are not able to assay interactions between clotting factors, blood cell elements and vascular endothelium. [8,9]. Moreover, they have been validated to monitor vitamin K antagonists and heparin therapy , thus they cannot predict and/or guide therapy in acute hemorrhages.[10] Current CCTs are also unable to predict thrombotic in acute leukemia [11]. Moreover, bleeding complications are quite uncommon in non-neoplastic thrombocytopenia, others factors must be

involved to explain an increased bleeding tendency in patients with acute leukemia under chemotherapy. Thromboelastography (TEG) is a global haemostatic 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. [12] During the last years, TEG has become a valuable tool in detecting coagulopathies and guiding hemostatic and transfusion therapies in different clinical settings including trauma care, cardiac surgery and liver transplantation [12-13]. Moreover, it is adopted to monitor hemorrhage and guide therapy during obstetric surgery, within the emergency intensive care units (ICU) [14,15]. The implementation of TEG offers several advantages considering that it is a fast and inexpensive assay, results can be available within five to ten minutes. Considering the advantages offered by TEG, its employment is growing-up in other contests and recently TEG has been adopted to predict thromboembolic events and hypercoagulability in patients with malignancies including hematologic malignancies.[16-17]

Ideally, TEG instrumentation is commonly available in ICU and in cardiology surgery units, thus it can be easily accessible. However, to date there has been limited research investigating the use of TEG in patients with acute leukemia mainly focused on predicting bleeding risk in presence of thrombocytopenia. [18-26]

Since a clinical perspective, the employment of TEG to evaluate global hemostasis may provide a better in-vivo assessment of the haemostatic balance in patients affected by acute leukemia during the different phases of the disease. The primary aim of this study was to assess with TEG the coagulation balance in patients with acute leukemia, from diagnosis to the end of chemotherapy.

4. Acute leukemia

Acute leukemia (AL) is a proliferation of immature bone marrow-derived cells (blasts) that may also involve peripheral blood or solid organs. As a result, there is an accumulation of leukemic blasts or immature forms in the bone marrow, peripheral blood, and occasionally in other tissues, with a variable reduction in the production of normal red blood cells, platelets, and mature granulocytes. The percentage of bone marrow blast cells required for a diagnosis of acute leukemia is 20% for many leukemia types, and do not require any minimum blast cell percentage when certain morphologic and cytogenetic features are present. The diagnosis and classification of AL is based on morphology, immunophenotype, and cytogenetic/molecular features of blood, bone marrow, lymph node, or other tissue. The first attempt at classifying AL was the French American British (FAB) Cooperative Group morphological criteria that divided acute myeloid leukemia (AML) into 8 subtypes (M0 to M7) and acute lymphoblastic leukemia (ALL) into 3 subtypes (L1 to L3) according to degree of maturation. The FAB classification system can be useful, but it does not take into account many of the factors that are now known to affect prognosis. The World Health Organization (WHO) system, most recently updated in 2016, includes some of these factors to try to better classify AL. [19]

The *WHO 2016* classification distinguishes six groups of AML: (1) AML with recurrent genetic abnormalities, (2) AML with myelodysplasia-related changes, (3) Therapy-related myeloid neoplasms, (4) AML Not Otherwise Specified, (5) Myeloid sarcoma, and (6) Myeloid proliferations related to Down syndrome. As regard ALL, in 1997 *WHO* identified three types of ALL: B lymphoblastic, T lymphoblastic and Burkitt-cell Leukemia. Later revised in 2008, Burkitt-cell Leukemia was eliminated as it is no longer seen as a separate entity from Burkitt Lymphoma, and B-lymphoblastic leukemia was divided into two subtypes: B-ALL with

recurrent genetic abnormalities and B-ALL not otherwise specified. B-ALL with recurrent genetic abnormalities is further delineated based on the specific chromosomal rearrangement present. In 2016, *WHO classification* added two new provisional entities to the list of recurrent genetic abnormalities and the hypodiploid was redefined as either low hypodiploid or hypodiploid with TP53 mutations. In adults, B-cell ALL accounts for 75% of cases while T-cell ALL comprises the remaining cases. Immunophenotyping is required to distinguish T-cell ALL from B-cell ALL.

4.1Acute Myeloid Leukemia (AML)

4.1.1 Epidemiology

Acute myeloid leukemia (AML, also known as acute myelogenous leukemia) consists of a group of relatively well-defined hematopoietic neoplasms characterized by a clonal proliferation of myeloid precursors with a reduced capacity to differentiate into more mature cellular elements. AML is the most common acute leukemia in adults, accounting for ~ 80 percent of cases in this group. The incidence of AML increases with age, from ~ 1.3 per 100 000 population in patients less than 65 years old, to 12.2 cases per 100 000 population in those over 65 years. Although advances in the treatment of AML have led to significant improvements in outcomes for younger patients, prognosis in the elderly who account for the majority of new cases remains poor. Even with current treatments, as much as 70% of patients 65 years or older will die of their disease within 1 year of diagnosis. [20] AML has been associated with environmental factors (eg, exposure to chemicals, radiation, tobacco, chemotherapy) and genetic abnormalities (eg, trisomy 21; Fanconi anemia; Bloom's syndrome; familial mutations of *CEBPA*, *DDX41*, *RUNX1*). In some patients, evolution to AML is preceded by evidence of

clonal hematopoiesis manifest as myelodysplastic syndrome, myeloproliferative neoplasms, paroxysmal nocturnal hemoglobinuria, and aplastic anemia.

4.1.2 Signs and symptoms

The increased production of malignant cells, along with a reduction in these mature elements, results in a variety of systemic consequences including anemia, bleeding, and an increased risk of infection. The signs and symptoms reflect the development of anemia; however, weakness, loss of sense of well-being, and fatigue on exertion can be disproportionate to the severity of anemia. Easy bruising, petechiae, epistaxis, gingival bleeding, conjunctival hemorrhages, and prolonged bleeding from skin injuries reflect thrombocytopenia and are frequent early manifestations of the disease. Very infrequently, gastrointestinal, genitourinary, bronchopulmonary, or central nervous system (CNS) bleeding occurs at the onset of disease. Pustules or other minor pyogenic infections of the skin and of minor cuts or wounds are most common. With intensification of neutropenia and monocytopenia after chemotherapy, major bacterial, fungal, or viral infections become more frequent. Anorexia and weight loss are frequent findings. Fever is present in many patients at the time of diagnosis. Palpable splenomegaly or hepatomegaly occurs in approximately one-quarter of patients. Lymphadenopathy is extremely uncommon, except in the monocytic variant of AML. [2021]

4.1.3 Prognostic factors

The diagnosis is made by the presence of ≥20% blasts in the peripheral blood or in the bone marrow, or through the presence of unique genetic abnormalities found in the bone marrow regardless of blast count [t(8;21), inv(16), or t(15;17)]. Accurate assessment of prognosis is central to the management of AML. By stratifying patients according to their risk

of treatment resistance or treatment-related mortality (TRM), prognostic factors help guide the physician in deciding between standard or increased treatment intensity, consolidation chemotherapy or allogenic hematopoietic stem cell transplant, or more fundamentally in choosing between established or investigational therapies. Among clinical factors, increased age and poor performance status are both associated with lower rates of complete remission (CR) and decreased overall survival (OS). Age and performance status at diagnosis similarly help to predict the risk of TRM, although multivariate model analyses suggest that other variables such as platelet count, serum creatinine or albumin rather than age itself account for most of the increased risk of TRM seen in older patients. Therapy-related AML and AML associated with a prior hematological malignancy also carry a significantly poorer prognosis. Although clinical factors have an important role in guiding therapy, cytogenetic changes constitute the single strongest prognostic factor for CR and OS in AML. AML is further classified into three prognostic risk groups: favorable, intermediate, and adverse. These are based on both cytogenetics and relatively recent recognition of molecular diseases subsets that are distinct from the contribution of cytogenetic risk. These newly recognized molecular subsets have different responses to standard therapeutics. The prognostic groups predict the response to standard therapy and survival in one large retrospective analysis of patients under the age of 55, the overall survival rate at 5 years was 44%, however when broken down by risk profile the overall survival rates were 64%, 41%, and 11% for favorable, intermediate, and adverse risk respectively. The overall survival decreases when older adults are included but the stratification of survival remains constant.[22]

4.1.4 Therapy

The general approach to current therapy has not changed substantially in recent years and usual treatment of AML includes an initial program termed the induction phase. The main treatment for most types of AML is chemotherapy, sometimes along with a targeted therapy drug. This might be followed by a stem cell transplant. Other drugs (besides standard chemotherapy drugs) may be used to treat people with acute promyelocytic leukemia (APL). Choice of initial induction treatment depends on functional status of the patient, biological status of the disease (best measured by prognostic risk groups and recently recognized molecular profile of leukemia cells) and goals of the patient. Initial assessment evaluates whether a patient is considered a candidate for intensive induction chemotherapy.

The two commonly used induction therapies in acute myeloid leukemia include 1) Cytotoxic chemotherapy with or without targeted therapies and 2) Hypomethylating agents with or without targeted therapies. [24]

A combination of anthracycline and cytarabine has been the standard induction therapy since 1973. A now classic standard induction regimen is cytarabine 100 mg/m2 daily by continuous infusion on days 1 through 7 and daunorubicin at 45 to 90 mg/m2 on days 1 through 3 (commonly referred to as "7+3" regimens).[25] This induction therapy, leads to complete disease response in up to 80% of patients with favorable risk disease and 50–60% complete response in those with intermediate adverse risk disease.

The outcomes have improved with addition of various targeted drugs to the traditional 7+3 induction chemotherapy in the favorable and intermediate risk groups. Gemtuzumab ozogomycin (GO) is a monoclonal antibody against CD-33 (a protein that is expressed in myeloid leukemia cells). Addition of GO to standard chemotherapy in patients with favorable and intermediate risk disease decreases the risk of relapse and in some studies improves OS. [26]

Midostaurin is an oral multi-targeted tyrosine kinase inhibitor (TKI) active in patients with a FLT3 mutation. FLT3 mutations initiate oncogenic signal transduction in about 25–30% of patients with AML. Addition of midostaurin to standard 7+3 chemotherapy in patients with FLT3 mutation has improved survival, from a median of 25 to 74 months. [27] Intensive induction therapy has many complications, but myelosuppresion, which manifests as neutropenia, thrombocytopenia and anemia, is one of the most important and mainly responsible for the mortality of AML patients. [28] Once a remission is obtained, further treatment is indicated to preserve the remission state.

4.2Acute lymphoblastic leukemia (ALL)

4.2.1 Epidemiology

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extramedullary sites. The estimated annual incidence of ALL-worldwide is 1 to 5 cases/100,000 population, and more than two-thirds of cases of ALL are B cell phenotype. While 80% of ALL occurs in children, it represents a devastating disease when it occurs in adults. Within the United States, the incidence of ALL is estimated at 1.6 per 100 000 population. In 2016 alone, an estimated 6590 new cases were diagnosed, with over 1400 deaths due to ALL (American Cancer Society). The incidence of ALL follows a bimodal distribution, with the first peak occurring in childhood and a second peak occurring around the age of 50.

4.2.2 Prognostic factors

Prognosis in B-ALL/LBL is associated with age, white blood cell count, race, male sex, and cytogenetic/genetic features. Increasing age and elevated white blood cell count at diagnosis, defined as 30×10^9 for B-ALL or $100x10^9$ for T-ALL, portend a worsening prognosis. The cytogenetic aberration with the greatest impact on prognosis and treatment is the presence of the Philadelphia chromosome, t(9;22). The prevalence of t(9;22) in adult ALL can range from 15–50% and increases with age. Ph positivity has implications both in terms of prognosis and for treatment. In addition to disease characteristics at the outset, it has long been recognized that response to initial therapy predicts outcome.

4.2.3 Signs and symptoms

Most of the clinical manifestations of ALL reflect the accumulation of malignant, poorly differentiated lymphoid cells within the bone marrow, peripheral blood, and, extramedullary sites. Presentation can be nonspecific, with a combination of constitutional symptoms and signs of bone marrow failure (anemia, thrombocytopenia, leukopenia). Common symptoms include 'B symptoms' (fever, weight loss, night sweats), easy bleeding or bruising, fatigue, dyspnea and infection. Involvement of extramedullary sites commonly occurs and can cause lymphadenopathy, splenomegaly or hepatomegaly in 20% of patients. CNS involvement at time of diagnosis occurs in 5–8% of patients and present most commonly as cranial nerve deficits or meningismus. T-cell ALL also may present with a mediastinal mass.[29]

4.2.4 Therapy

The structure of treatment of adult ALL has been adapted from pediatric protocols. Unfortunately, while long-term survival approaches 90% for standard-risk pediatric ALL, the success rate is much more modest in adults. Chemotherapy consists of induction, consolidation and long-term maintenance, with CNS prophylaxis given at intervals throughout therapy. The goal of induction therapy is to achieve complete remission and to restore normal hematopoiesis. The backbone of induction therapy typically includes vincristine, corticosteroids and an anthracycline. The role of L-asparaginase, while standard in pediatric protocols, is a challenge in adults at times due to the increased rate of adverse events. [30] L-asparaginase decreases plasminogen, fibrinogen, and antithrombin, resulting in impaired thrombin inhibition, which may contribute to the asparaginase related dose-limiting toxicity. Changes in antithrombin (AT) and fibrinogen during induction chemotherapy with L-asparaginase were reported in a retrospective study of 214 adult patients with ALL. While dose intensification strategies have led to a significant improvement in outcomes for pediatric patients, prognosis for the elderly remains very poor.[29] Despite a high rate of response to induction chemotherapy, only 30–40% of adult patients with ALL will achieve long-term remission. [29]

Special consideration must be made in the treatment of Ph-positive ALL. Historically, Ph-positive ALL was a very bad player with 5-year survival 5–20% and Allo-SCT being the only chance for cure. The advent of TKIs marked a turning point in the treatment of Ph-positive ALL.[31]

4.3 Bleeding and thrombosis in acute leukemia

The course of acute leukemia may be accompanied by various abnormalities of hemostasis, from severe thrombocytopenia to subclinical activation of clotting or chronic disseminated intravascular coagulation (DIC). Clinical manifestations can range from localized venous and/or arterial thrombosis to diffuse life-threatening bleeding. The incidence of these complications varies according to the type of leukemia and the phase of treatment.

In adult patients with AL the 6%–7% of whom experience serious to life-threatening bleeding: ie, WHO grades 3 and 4; resulting in mortality or lasting morbidity, or requiring medical intervention. Intracranial hemorrhage (ICH) is the second most common complication, after infective complications. [32]

Even if severe thrombocytopenia is considered a key factor, the definite cause of ICH in patients with hematological malignancies is inconclusive. Chemotherapy related endothelial injury and reduction of coagulation factors also could play a role in the pathogenesis of ICH.[1,6]

To prevent serious and spontaneous bleeding, prophylactic platelet transfusion is standard of care for patients affected by AL when showed a low platelet counts.

Currently, these platelet transfusions are given prophylactically when the platelet count is below 10×10^9/L. Even if the platelet count is the main trigger for prophylactic platelet transfusions, it per se is poorly correlated with bleeding risk, since a significant percentage of bleeding episodes might occur at relatively high platelet counts and despite platelet transfusions.[33]

On the other hand, although venous thromboembolism (VTE) has been considered less characteristic than hemorrhage in patients with AL, recent studies suggest that thrombosis is more common than previously appreciated [34].

The reasons for this increase in thrombotic risk are complex and poorly understood. The pathogenesis of VTE in the context of AL is multifactorial depending on: 1) pro-coagulant

properties of leukemic cells; 2) patients characteristics; 3) therapeutic interventions; 4) presence of central venous catheter (CVC) and of septic complications. [6,16]

Among the postulated mechanisms for chemotherapy-related thrombosis are: (1) release of procoagulant products from apoptotic malignant cells; (2) drug toxicity directed at the vascular endothelium; (3) direct induction of monocyte or tumor-cell Tissue-Factor (TF); and, (4) a decrease in physiological anticoagulants. [1]

VTE incidence in AL ranges from about 2% up to 12%[5, 35-36]. Ku et al. [5] have observed a 2-year VTE cumulative incidence of 5.2% in AML and 4.5% in ALL, in a cohort study on 7876 patients with acute leukemia, mainly during the first month of diagnosis: age, comorbidities and CVC were reported as the most frequent associated risk factors for VTE.

De Stefano et al. [35] evaluated the risk of VTE in a cohort of 379 adult patients with a newly diagnosed AL; overall, VTE episodes occurred in 19 (5%) patients and in 13 cases of entire population (3.4%), it was the presenting manifestation. In particular, VTE at diagnosis was observed in 1.4% of ALL, 9.6% of APL and 3.2% of other AML. Moreover, patients treated with L-asparaginase had a 4.9 fold increased risk of thrombosis (95%CI: 1.5–16).

Death rate due to thrombosis was 0.8% and, differently from AML, in ALL the occurrence of VTE increase of 40% the risk of dying within 1 year. Estimated incidence of VTE in children with ALL, derived from prospective studies, range from 3% to 36.7%[2] . A meta-analysis of 17 studies showed a 5.2%, rate of thrombosis in pediatric patients with ALL mainly during induction therapy with L-asparaginase[36]. A similar trend (incidence rate of 5.9%) was observed in adults with ALL from 13 published prospective studies including 323 patients [37]. Most VTEs occurred within 3 months from diagnosis; however, most of them were upper extremity CVC related deep vein thrombosis and occurred mainly in ALL.

Since patients with acute leukemia have a high risk of hemorrhage, the administration of anticoagulant therapy and/or prophylaxis for VTE poses serious challenges.[38,39]

5. Conventional coagulation tests

A model of the plasmatic coagulation distinguishes an extrinsic pathway, which starts with tissue factor/factor VII activation, an intrinsic pathway that is activated by contact factors, and a common pathway comprising the coagulation factors II, V and X. The coagulation cascade is a tightly controlled series of enzymatic and cell-based reactions, designed to generate thrombin, which converts fibrinogen to fibrin, and requires co-factors such as phospholipids and calcium ions. It is usually subdivided into an initiation phase, an amplification phase and a propagation phase. [40]

Conventional coagulation tests (CCTs) assess blood coagulation and include prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), platelet count, fibrinogen concentration, D-dimer level. PT, described first by Quick in 1935, is still referred as "Quick's PT" or "Quick value". Originally developed to determine PT (factor II of the coagulation cascade), it is now clear that it depends especially on factor VII together with the factors X, V, II and fibrinogen. [41] Changes over time have made the test more specific for the vitamin K dependent clotting factors (VII, X, V and II). The initiation of clotting, specifically of the intrinsic pathway, is naturally triggered by negatively charged phospholipid surfaces (platelet). This reaction is artificially replaced by contact phase activators. In the past through cephalin, currently through minerals as kaolin or celite and more rarely silica or glass dust. The APTT depends initially on factor XII and XI and reflects the intrinsic pathway, especially factors VIII, IX and XI. Originally designed in 1953 as a simple one-stage test for hemophilia. At that time, the test was activated by cephalin (phospholipid) and consequently replaced by kaolin (mineral) to optimize the contact phase.[42]

These tests are usually used for the clinical diagnosis of coagulopathy, to monitor anticoagulation therapy, and to assist in the treatment of bleeding episodes.[11] Despite being very effective for specific clinical needs, such as anticoagulation monitoring, they have limitations. Their main disadvantage in circumstances of acute major bleeding is long turnaround time.

These tests are plasma-based assays evaluating the thrombin generation determined by pro-coagulation factors but ignore the interaction of the coagulation factors with blood cell elements and the vascular endothelium.[43] Furthermore, they are enable to assess PLT function, the activity of the fibrinolytic system and endogenous anticoagulant factors. They reflect the situation in vivo only partially, and may be influenced by a variety of pre-analytical factors.

Similarly, fibrinogen according to Clauss may overestimate the fibrinogen concentration in the presence of hydroxyethyl starch.

Platelet concentration, easily measured as part of a complete blood count, does not necessarily reflect their function, especially in the presence of elements known to affect platelet reactivity, antiplatelet agents, uremia, malignancy, or alcohol intake. VTE is another common and serious condition that is associated with abnormal blood coagulation. In these cases, systemic hypercoagulability shifts the body's homeostatic mechanisms toward a pro-thrombotic state. Common coagulation testing has not been shown to predict such events, and in many cases, even a detailed hypercoagulability investigation fails to identify an underlying disorder.

6. Thromboelastography (TEG)

Thromboelastography (TEG) is a point of care (POC) viscoelastic test of hemostasis which allows measurement of haemostatic function, from the beginning of clot formation to the end of fibrinolysis, including initiation, formation, and stabilization of the clot.

TEG was developed and first described by Hartert at the University of Heidelberg (Germany) in 1948 and its use in clinical practice was not described until many years later (1960s) when physicians performing early liver transplantation surgeries described use of TEG to detect and treat fibrinolysis occurring after reperfusion of the transplanted liver. Successively in 1990s, TEG was demonstrated to be useful in cardiac surgery. [44,45] TEG is a laboratory method that demonstrates the interactions among the blood cells and their biochemical characteristics by means of a graphic representation. The principle of this in vitro test is to detect and quantify dynamic changes of the viscoelastic properties of a blood sample during clotting under low shear stress. The test is performed in a specially designed system called a thromboelastograph (**Figure 1**). The system consists of two chambers simultaneously examining a blood sample in duplicate to reduce the risk of sampling and measurement errors. Each chamber consists of a plastic plate, upon which is placed a disposable cup, attached via mechanical arms to the TEG device. Whole blood is placed in the disposable cup, and after activation of clotting (usually with kaolin), the plastic plate and cup are moved up into contact with the pin. Induced pin movement is recorded and changes measured as a function of time. At this point, the TEG tracing is initiated. After the tracing is initiated, the mechanical arms on the TEG instrument rotate the cup at a fixed frequency. Initially the pin, attached to the instrument via a torsion wire, will be motionless in the cup of whole blood as there is no means for the liquid whole blood to exert force on the pin. As whole blood coagulates, the viscosity of the blood increases and at the same time the pin becomes cross-linked to the cup via fibrin and platelet interactions.

These gradually increasing viscoelastic mechanical properties of the blood are reflective of the developing 3-dimensional fibrin mesh and platelet components of the clot. The greater the viscoelasticity of the clot, the higher the amplitude of the pin motion. The thromboelastogram (**Figure 2**) is a graphical image of the recorded amplitude of movement of the pin as a function of time. Analytical software measures and quantifies these changes. Whole blood for TEG analysis is usually collected in sodium citrated VacutainerVR® tubes and then recitrated when the whole blood sample is pipetted into the sample cup. Non-citrated whole blood (native blood TEG) can also be tested, but it must be used immediately. The test and reagents used are at room temperature. A volume of 340 uL of citrated blood is pipetted to the study cup, recalcified by the addition of 20 uL of 0.2M calcium chloride and then activated with a kaolin-cephalin reagent. Cephalins, or phosphatidylethanolamines, are a class of phospholipids commonly present in membranes of human cells. They are an important cofactor of the coagulation cascade which enables the assembly of tenase and prothrombinase complexes on the surface of platelets which are critical for thrombin generation. Kaolin is a mineral, primarily composed of hydrated aluminum silicate, which is a negatively charged molecule that can initiate the intrinsic coagulation pathway by activating Factor XII. Precise proportioning of the blood and kaolin-cephalin reagent is important for accurate and reproducible TEG results. The parameters of the thromboelastogram include the reaction (R) time, the time from initiation of the TEG tracing until torsion is exerted on the pin/wire assembly. R time largely reflects the adequacy of coagulation factors (similar to what is measured in the laboratory by PT and aPTT). The K (time to reach 20 mm clot amplitude) and the angle (angle formed by line tangent to clot curve, **Fig. 2**) reflect clot kinetics and are determined by a number of factors including thrombin generation and fibrinogen concentration. The maximum amplitude (MA) is the maximum distance traveled by the cross-linked cup/pin, which reflects maximum clot strength and is

largely a function of platelet function or activity. Finally, the Lysis30 or Lysis60 is the percent decrease in area under the TEG curve (from MA) over 30 or 60 min. All of these parameters can be compared to normal or reference values to detect either clotting factor deficiencies (increased R time), fibrinogen deficiencies (decreased angle), platelet function defects (decreased MA), or excess fibrinolysis (Lys30 or Lys60 outside of reference values). The TEG tracing thus provides an overview of multiple coagulation processes and allows detection of variables that affect conventional coagulation tests, as well as factors that cannot be easily measured by conventional testing as clot kinetics, which is dependent upon both thrombin generation and fibrinogen concentration and platelet function. Another advantage of TEG over conventional coagulation testing is that the TEG R time is significantly more sensitive to clotting factor deficiencies or the presence of heparin than are conventional tests such as APTT or PT. A special cup containing heparinase can be used to determine whether the presence of heparin (administered therapeutically or through accidental sample contamination) has influenced the TEG parameters.[46]

TEG analysis of patients with a history of thromboembolic complications showed shorter R values and accelerated clot propagation compared to healthy reference subjects.[15] The TEG may be helpful in screening for hypercoagulable states. During the last years, these tests have been shown to be suitable for detecting and treating coagulopathy in trauma care, cardiac surgery and liver transplantation. As a point of care device, it may also monitor hemorrhage and control therapy during obstetric surgery, at the emergency department, and at the intensive care unit. [47]

Furthermore, TEG is recommended by guidelines to help detect, manage and monitor hemostasis in cardiac surgery patients. Another potential application of TEG is to improve diagnosis, prevention, and treatment of patients with VTE.

The most appropriate evidence-based practice to prevent VTE is to stratify patients based on the VTE risk using one of the risk prediction models elaborated for surgical and medical patients. None of these models includes conventional blood coagulation tests since they do not predict VTE. A number of initial reports suggest that TEG may be a useful tool to help with risk stratification.[48,16] To date, there have been reported cases of the utilization of TEG to predict thromboembolic events and hypercoagulability in patients with hematologic malignancies.

7. Materials and Methods

7.1 Population

A prospective observational pilot study was performed with the primary aim of assessing with TEG the haemostatic balance in patients with AL, from diagnosis to the end of chemotherapy. The secondary aim was a specifically analyze of TEG profiles in patients with clinically relevant thrombotic and/or haemorrhagic complications. The Ethics Committee of University Hospital of Palermo (number of protocol 04/2018) approved this study. Informed consent was collected from all participants according to the Helsinki declaration. Patients affected by AL, reporting at the Unit of Hematology of University Hospital of Palermo, fulfilling the inclusion criteria and providing informed consent, were consecutively enrolled from the beginning of May 2018 to the end of December 2019. Eligible patients were ≥18 years of age, with a new confirmed diagnosis of AL {acute myeloid leukemia (AML) and acute lymphoid (ALL)}. Diagnosis of AL was based on conventional morphocytochemical criteria, immunophenotyping, and cytogenetic and/or molecular tests according to WHO classification.[19] The exclusion criteria were as follows: diagnosis of Acute Promyelocytic Leukemia (APL), known coagulation disorders, known liver diseases, concomitant solid neoplasms and regular use of anticoagulants. All the enrolled subjects received treatment regimens in accordance with international guidelines from the European LeukemiaNet (ELN) for disease type, age and comorbidities [22]. According to standard practice, when the morning platelet count was $<10x10^9$/mL, patients received prophylactic transfusion with one batch of platelet concentrate; while target of Hb for transfusion was usually 8.0 g/dl and 8.5 g/dl in selected cases (active infection like acute pneumonia and known cardiovascular comorbidities). Number of platelet (PLT) and red blood cell (RBC) transfusion units received during the study

was recorded. Assessment of blood count cells (CBC), conventional coagulation tests (CCTs) including PT, a-PTT, DD, FBG and TEG were made at the following time points: 1) Diagnosis of AL (T0); 2) During the first cycle of CHT (T1); 3) During myelosuppression T2 (PLT $\leq$ $30x10^9$/L, absolute neutrophil count < 1000/mmc); 4) At the end of the first cycle of CHT (T3). Baseline characteristics (age, comorbidities, type of leukemia, treatment received) were recorded in a dedicated database accessible to study staff and protected by password. Coagulation samples were collected at least 24 hours (h) after PLT or RBC transfusion, in absence of any concomitant anticoagulant treatment. Patients were followed-up for bleeding and thrombotic episodes daily up to the time of hospital discharge or death. Bleeding was graded 1 to 4 according to the WHO criteria. Thrombosis included VTE, superficial venous (SVT), arterial thrombosis, all objectively diagnosed and confirmed using standard diagnostic testing (i.e. ultrasound and computed tomography).

7.2 Thromboelastography (TEG)

The TEG was performed on citrated whole blood samples within 2 hours after blood collection by a Haemoscope Thrombolastograph® Haemostasis Analyzer model 5000. Testing carried out by the same-trained biomedical scientists using citrated blood samples (340 ml) that were recalcified with 20 ml of 0.2 M CaCl2. The TEG parameters reaction time (R, min), α (alpha-angle, °), kinetic time (K, min), maximum amplitude (MA, mm) and coagulation index (CI), were recorded. The normal reference ranges, defined by internal control, were considered as R 9-27 min, K 2-9 min, α 22°-58°, MA 44-64 mm, CI -3;+3. Thrombin generation (TG) was indirectly calculated by an algorithm of the instrument and normal range was considered 494-824 mm/min. Quality control was maintained per manufacturer's instructions.

7.3 Blood count and coagulation tests

Samples underwent hematology test and assayed using COBAS INTEGRA 800 biochemical analyzer (Roche, Basel, Switzerland). APTTs and PTs were performed using on the automated coagulometer ACL TOP 750 series (Instrumentation Laboratory, Werfen) and FBG was assayed using the Clauss method.
The reagents and methodology were used according to the manufacturer's instructions. Platelet - poor plasma (PPP) was obtained by centrifugation of citrated blood at 10000 g for 5 min and then at 1500 g for 3 min. All samples were collected at the appropriate time point and CBC, fibrinogen measurements were performed within 2 hours after blood collection. All other tests were performed batchwise in stored PPP (−80° C), which was frozen in aliquots within 2 hours after blood collection. Normal reference ranges were taken from the reagents instructions: PT-ratio 0.8-1.2, APTT 24-36 sec, fibrinogen 150-450 mg/dl, DD 10-250 μg/L.

7.4 Statistics

Continuous variables are presented as mean ± standard deviation (SD) for normally distributed traits while categorical variables are expressed as counts and percentages. To evaluate the behavior of TEG parameters before, during and after chemotherapy the paired *t* test was used. It has been analyzed the difference between diagnosis and chemotherapy (T1 vs T0), during and after chemotherapy (T1 vs T3). Moreover, in patients who experienced myelosuppression we analyzed difference between this phase and chemotherapy (T2 vs T3) with the paired *t* test. We used Pearson's correlation to evaluate the relationship between TEG parameters and CCTs and between TEG parameters and the values of CBC. Two-sided P values

of <.05 were considered statistically significant. Statistical analysis and graphs were performed using software Open Source R.

8. Results

8.1 Patients

Forty consecutive patients were enrolled: 31 with newly diagnosed AML and 9 with ALL. As for disease molecular characteristics, we distinguished AML-NOS in 27 patients, FLT3-mutation was found in 3 patients, AML with mutated NPM1 and AML with myelodysplasia-related changes in two and one patient, respectively. AML was secondary to Polycythemia Vera (PV) in one patient; one patient had a Philadelphia Chromosome positive acute lymphoblastic leukemia (Ph+ALL). Population consisted of 23 males (57%) and 17 females (43%) with a mean age of 62 years (range 18-80) and mean time of follow-up lasting 31 days (range of 7-69). **Table 1** shows clinical and demographic characteristics of the enrolled patients.

TEG at T0 was performed in all the enrolled patients (N=40), at T1 TEG was available for 29 patients at T2 for 25 subjects. TEG at T1 was not carried out in 11 patients for the following reasons: 5 patients moved to another haematological center, 3 patients were not treated with CHT, receiving exclusively supportive therapy at home, 3 patients died after diagnosis, before the begging of CHT, for infective complications. T2 was not performed in four patients because three of them died during CHT and one received a continuative therapy with hydroxyurea. TEG during phase after CHT (T3) was carried out in fourteen patients with AML receiving high dose of CHT. Treatments administered were inductive chemotherapy (n=18), hypomethylating agents (HP) (n=9), tyrosine kinase inhibitor (TKI) (n=1) and hydroxyurea (n=1). Induction regimens adopted were cytarabine plus daunorubicine (7 plus 3) and fludarabine, cytarabine

and filgrastim (FLAG) for AML and vincristine, prednisone, cyclophosphamide, doxorubicin and L-Asparaginase for ALL. All cases with FLT3-mutated AML received also midostaurin. Primary antithrombotic prophylaxis was not administered in any enrolled patient.

8.2 Hemostasis in acute leukemia patients at diagnosis

TEG results at each time-point are summarized in **Table 2, Figure 3**. TEG parameters at baseline, showing the highest proportion of values outside interval reference range were R and MA, they resulted reduced in 47% and 35% of patients, respectively TG and K were also reduced (25% and 27% of results below reference intervals) while alpha-angle exceeded the normal range level in 14%. The mean PLT count was $66x10^9$/L (SD$\pm$$67x10^9$/L) and it was positively related to MA by Pearson coefficients: 0.69 ($p < 0.05$). In addition, a strong correlation was observed between MA and alpha-angle, (*Pearson's coefficient* 0.75, $p < 0.05$). As regard CCTs, mean values of APTT, PT/INR were within the normal reference range, while FBG was above the normal range in 11 patients and D-dimer levels were substantially increased in almost all patients. There was no significant correlation between CCTs and TEG parameters at the time of diagnosis. **Table 3** summarizes parameters of CBC and CCTs.

8.3 Haemostasis in acute leukaemia patients during chemotherapy

At T1, TEG parameters, analyzed in 29 patients, shifted toward a hypocoagulability state when compared to T0: MA (49 mm vs 41 mm; $p< 0.05$), α (50° vs 44°, $p <0.05$) and TG (604 mm/min vs 531 mm/min, $p<0.05$) were decreased while K and CI were increased (K, 3.2 vs 4.7 seconds, $p<0.05$; CI -0.15 mm to -0.22 mm, $p>0.05$). Otherwise R was shorter than T0 (9.3 vs 8.6, $p>0.05$). As regard CCTs, we found no significant differences compared to T0 and no correlation with global hemostasis test. Only the value of DD level appeared reduced during CHT ($p>0.05$). The value of PLT count was moderately reduced in comparison toT0 (55 $\pm$ 51

x10^9/L) and it was still positively correlated with MA (*Pearson's coefficient* 0.76, $p<0.05$). Finally, CBC parameters (Hb, Hct, Wbc) were significantly lower than at T0 ($p<0.05$).

8.4 Haemostasis in acute leukaemia during myelosuppression

TEG was performed in fourteen patients during the myelosuppresion phase (T2) after myeloablative treatment and compared to the other time-points **(Table 2).** In this phase, all TEG parameters were significantly shifted to hypocoagulable state in comparison to T1, in details: R and K were increased (R, 12 seconds vs 8.6 seconds, $p<0.05$; K, 7.8 vs 4.7 seconds, $p<0.05$) while alpha-angle and MA were decreased (α, 29° vs 44°, $p<0.05$; MA, 25 mm vs 41 mm; $p<0.05$). TG and CI were reduced (TG, 467 mm/min vs 531 mm/min, $p<0.05$; CI -1.95 mm to -0.22 mm, $p<0.05$). As expected, a significant reduction in all CBC parameters was detected with a severe thrombocytopenia. Platelet count was significantly decreased ($p < 0.01$), from 55 x10^9 /L $\pm$ 51 (T1) to 4.7 x10^9 /L $\pm$ 4.0 x 10^9/L (mean $\pm$ SD). Furthermore, CCTs did not show a significant correlation with TEG during myelosuppression. Only DD levels resulted reduced at T2 (DD, 1469 vs 2125 μg/L).

8.5 Haemostasis in acute leukemia patients after chemotherapy

At T3, alpha-angle and MA were significantly wider than at T0 (α 61° vs 50°; MA 59 mm vs 50 mm; $p<0.05$) and TG increased (735 mm/min vs 604 mm/min, $p<0.05$). K was slightly shorter ($p> 0.05$) and CI moderately increased. TEG parameters at T3 appeared significantly modified also in comparison to T1($p<0.05$) : α, 61° vs 44°; MA, 59 mm vs 44 mm; K 2.5 seconds vs 4.7 seconds, TG, 735 mm/min vs 531 mm/min; CI 0.69 mm to -0.22 mm). R-time was not significantly reduced compared to T0 and T1 (Figure 1). We found no significant changes in terms of CCTs and of their relationship with TEG parameters.

Platelets count was markedly increased compared to all the other time-points (T0, T1 and T2). At this time point persisted relationship between PLT and MA ($p<0.05$) as previous seen in the others. The values of Hb and Wbc, including absolute neutrophil count (ANC), were significantly increased ($p<0.05$) when compared to all the other time-points. Additionally, ANC showed a significant negative effect on K (*Pearson's coefficient* -0.75, $p<0.05$) and on R (*Pearson's coefficient* -0.65, $p<0.05$), and no impact on MA, angle and CI. TG appeared positively influenced by ANC (*Pearson's coefficient* -0.82, $p<0.05$).

8.6 Bleeding and thrombotic complications within the course of AL

Among 40 patients sixteen (40%) had hemorrhagic symptoms: 4 had WHO grade 4 bleedings, including central nervous system (CNS) hemorrhage (n=2), bleeding secondary to ruptured spleen (n=1) and 1 sub retinal hemorrhage; 2 had WHO grade 3, including epistaxis; 9 had WHO grade 2, including hematuria (n=2), skin petechiae (n=4), ecchymosis (n=1) and gum bleeding (n=1). Except one CNS-hemorrhage occurring in a patient with ALL, all bleeding complications were reported in patients with AML. In three patients, symptoms were reported during CHT (T1) (1 on hydroxyurea and 2 on HP), in four patients at AL diagnosis (T0) and in nine during mylosuppresion (T2). Overall, five patients (12%) experienced thrombotic symptoms: 2 deep vein thrombosis (DVT), 1 CVC related thrombosis, 1 portal vein thrombosis (PVT) and 1 pulmonary embolism (EP). In detail, three events occurred in AML patients: PVT was diagnosed before CHT (T0) in a patient with AML post-PV, while the other two complications occurred during CHT (T1). Two thrombotic events occurred in two patients with ALL, 1 EP during induction therapy (T1) and the other (DVT) in post-CHT phase (T3). There were no significant differences in terms of CCTs and TEG results between patients

experiencing hemostatic complications (bleeding and thrombosis) and those without, at either T0, T1, T2 or T3. Similarly, when CCTs and TEG results were compared based on the type of complications (thrombosis or hemorrhages), no changes were found at each analyzed time point. However, all the enrolled patients experiencing significant bleeding showed a trend toward a hypocoaguable state on TEG at T0, with longer R-times (mean 10.5 vs 8.25, $p > 0.05$) and K-times (4.15 vs 3.21, $p > 0.05$), in comparison to non-bleeders. Interestingly, in the patient with AML and CNS hemorrhage, some parameters of TEG (MA 24 mm, CI -4.7, TG 341 mm/min) performed at T2, the day before symptoms onset, resulted decreased. With reference to thrombotic complications, in 4 (80%) patients with VTE, TEG at T0 showed K and R below the reference values. Overall, transfusion requirements, numbers of RBC (mean $\pm$ SD, 9.7 $\pm$ 4.7 vs 5.1 $\pm$ 3.1, $p<0.05$) and PLT units (mean $\pm$ SD, 5.2$\pm$ 3.2 vs 0.55 $\pm$ 2.8, $p<0.05$) transfused were significantly increased in symptomatic patients with bleeding and in those with thrombosis.

9. Discussion

TEG is a rapid and easy to handle tool, in contrast with other global tests requiring specialized centers, it is widely available in several hospitals. Our results showed that TEG is able to identify changes in the haemostatic state of patients with AL according to the course of the disease. At diagnosis, a large dispersion of TEG parameters was observed in newly diagnosed patients, with a wide variability from a hypocoagulable to a hypercoagulable state. Interestingly, in about half (47%) of leukemic patients, the most frequent sign of hypercoagulability was a shorter R-time, suggesting an underlying increase in coagulation factors plasma levels and activity. Indeed R-time can be considered, as PT/INR and a-PTT, a surrogate marker of clotting factor levels. [41] However, in the current study, PT/INR and a-PTT did not result 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 AL before treatment. [49] According to these findings, TEG at diagnosis confirms the coagulation activation frequently observed in neoplastic patients and underlines the heterogeneity and complexity of haemostatic alterations in patients with AL [34], thus requiring an individual determination of the thrombotic and/or haemorrhagic risks.

Comparing TEG parameters at all 4 time-points planned, we found that the most clinically and statistically significant changes emerged during and after cytoreductive therapy, including myelosuppression phase. TEG identifies a hypocoagulable state during chemotherapy, further exacerbated on myelosuppression phase. In details, our results detected a slower rate of clot formation time (K-time and alpha-angle) during CHT, when compared to the time of diagnosis. Kim et al. showed that significant determinants of the K value were FVII, PLT and fibrinogen. [50] However in this phase, platelets count resulted only moderately decreased respect to the diagnosis and fibrinogen levels were within the normal range. Considering that a decrease in FVII levels has been reported in hematological patients during CHT [51], we may suppose a reduction in FVII as a potential cause of hypocoagulability. On the other hand, the presence of shorter R-time could be in contrast with our hypothesis, however: - first this finding was not statistically significant; - second, it could mirror a concomitant endothelial cell activation and tissue factor up-regulation induced by CHT. [52,53] As regards the alpha-angle, it expresses the buildup and cross-linking of fibrin, thus needing sufficient amounts of fibrinogen, thrombin, and platelets. Thus, another potential mechanism responsible for TEG results may be an alteration of platelets-fibrinogen interactions, as suggested by a previous study in which an increased alpha-angle value was associated with bleeding in thrombocytopenic patients.[54]

TEG results suggest that in AL, during treatment, the risk of bleeding cannot be simply attributed to the degree of thrombocytopenia but involves coagulant activity and interactions

between platelets and coagulation factors. Comparing myelosuppression to CHT phase, we found that all TEG parameters significantly switched to hypocoagulation. In addition to the signs of hypocoagulability already seen on chemotherapy, patients showed slower clotting time (R-time), weaker clots (MA) and marked reduction in thrombin generation. Heubel-moenen et al. found similar findings in chemotherapy induced thrombocytopenia using t-PA ROTEM. [55] Previous studies have shown that platelets influence fibrin formation and clot structure[56, 57] suggesting that weakness of clots might be due to the low platelets count. However, even if a severe thrombocytopenia characterizes myelosuppression, according to the relatively uncommon hemorrhages in non-neoplastic severe thrombocytopenia, other mechanisms must be involved in determining an increased bleeding risk. A recent study suggests that the increased bleeding risk in thrombocytopenic cancer patients undergoing treatment could be due to an impaired platelets function, not counter balanced by a higher coagulation activity. [51]

Moreover, in another study from Kim et al., patients with thrombocytopenia and severe bleeding had low thrombin levels.[50] This is in line with our findings where a slower clot formation and reduced thrombin generation suggests the lack of a compensatory higher coagulant activity during myelosuppression. It is important to take into account that severe thrombocytopenia could affect TEG results. However, in the literature conflicting results on the relationship between R time at TEG and platelet-count are reported.[55, 58] In the current study, a relationship between these two variables was not found. Finally, all the changes in TEG at T3 (after CHT) revealed a hypercoagulative status when compared to the chemotherapy phase. These findings could mirror a restoration of the coagulation balance after a hypocoagulative state induced by treatment. On the other hand, the hypercoagulative status in T3 may indicate a residual hypercoagulable effect of CHT as previously shown in other haematological neoplasms [59] including acute promyelocytic leukemia (APL). [60].

Indeed, in patients affected by APL, treatment can convert a hypocoagulable state in a hypercoagulant condition. [60] Interestingly, at T3, ANC was negatively related with R and K and positively related to TG. There are several aspects to consider when interpreting these findings: reticulated platelets are immature platelets released in the bone marrow, representing an early marker of thrombopoietic regeneration after chemotherapy or haemopoietic stem cell transplantation.[61,62] Previous reports have shown immature platelets to have hyperexpressed haemostatic functions than mature or transfused platelets. [63, 64] In the context of these results, we suppose that activated immature platelets, increased after chemotherapy, may interact with a progressive growing ANC to induce degranualation [65] and procoagulant mediators realease,thus determining a hypercoagulative state. In a previous study on thirteen thrombocytopenic, haemato-oncologic patients, there was no significant correlation between white blood cells and R, in contrast to our results.[54] A possible explanation to the contradictory results may be that in our study, a significant relationship between ANC and R was present after chemotherapy and not during it. Concerning clinical complications, the incidence of VTE was 12% while hemorrhagic complications occurred in 40% of the enrolled patients. In our cohort, TEG parameters did not show any statistically significant difference in patients developing hemostatic symptoms and those who did not, at all the examined time points and for both type of complications. However, this could be due to the sample size of our study. It should be noticed that in 80% with VTE, TEG at T0 showed decreased K and R values, while patients having significant bleeding showed all a more hypocoaguable profile on TEG at T0 (longer R and K), than their non-bleeding counterparts. In addition, TEG performed the day before the onset of VTE symptoms, revealed signs of hypocoagulability (diminished MA, α, and TG) in the patient with CNS hemorrhage and in presence of PLT > 10 x 10^9 /L. This is in line with other studies, showing that TEG parameters perform better in discriminating between

patients having significant bleeding. Either alone [66] or in association with platelets count [67] than platelets count alone. Finally, in our study patients with clinical complications received an increased number of PLT and RBC units transfusion ($p<0.05$) in comparison to those without complications. Our findings are consistent with previous studies in which transfusions were associated with VTE, arterial thrombotic events (ATE) and in-hospital mortality in neoplastic patients. [68] A possible explanation is that anemia and severe thrombocytopenia are a surrogate marker of aggressive tumor biology, or for more intense cytoreductive treatments, related to complications. It should be noted that there was no correlation between any TEG parameter and conventional clotting test results in patients during all the four time-points analyzed, thus confirming that standard assays are not a valuable tool to monitor patients with AL. Our results suggest that TEG may be clinically useful to early detect changes in haemostatic status of patients with AL during treatment, where standard coagulation tests are known to be inadequate. Our findings confirm that, although AL is associated with a high risk of life-threating bleedings, VTE occurs in a not negligible percentage of cases (12%). Prophylaxis and therapy of VTE is still a challenging issue in patients with AL[38,39] and calls us to find tools able to predict thrombotic risk and drive therapeutic interventions. Additionally, TEG has shown the ability to detect prohemorrhagic features, besides thrombocytopenia and in presence of normal CCTs. Thus, TEG could contribute to guide transfusion and hemostatic therapy during the course and different phase of treatment in AL. Another important aspect to consider is that TEG is a fast and inexpensive assay with readily available results (within five to ten minutes). Moreover, it is an easy to handle instrumentation and ideally present in ICU and cardiology surgery units, thus it could be commonly available in hospitals without specialized coagulation centers. This study has several limitations. First, we studied patients treated with several chemotherapy regimens, but the number of enrolled subjects did not allow stratifying for type

of CHT. Second, we estimated TG levels by an algorithm of TEG and not directly by calibrated automated thrombogram method. Although this technique involves a time-consuming and complex process, which prevents its use for rapid diagnosis.[69] However, to our knowledge, this is the first study to have systematically evaluated global hemostasis test and coagulation parameters in patients with AL during different phases of disease, including myelosuppression and after chemotherapy.

Conclusion

In conclusion, our findings showed the ability of TEG to reveal complex and dynamic abnormalities in patients with AL according to the course of the disease and its treatment. Further studies will clarify the role of TEG as a valuable tool to define the hemostatic profile of patient and individualize the approach to transfusion, bleeding management and anticoagulant prophylaxis in patients with AL.

10.Tables and figures

Table 1. Patient Demographics and Clinical Characteristics

N° patients	40
Age — yr mean (range)	62 (18-80)
Sex F/M — no. (%)	17 (43)/23(57)
Time of follow-up –days (mean, range)	31 (7-69)
Diagnosis — no. (%) • AML-NOS • FLT3 mutated • AML-NMP1 mutated • AML post-PV • AML with MDS changes • ALL • ALL Ph+	 27 (67) 3 (11) 2 (5) 1 (2) 1 (2) 8 (20) 1(2)
Comorbidities—no. (%) • DM • Hypertension • Obesity • COPD • Hypothyroidism • Prostatic hypertrophy • Hypercholesterolemia • No comorbidity	 6 (15) 20(50) 3 (7) 8 (20) 4(10) 4(10) 5 (12) 5 (12)
N° patients	29
Treatment plan — no. (%) • TKI • Hydroxyurea • Cytarabine+daunorubicine (7+3) • FLAG • Vincristine,prednisone,cyclophosphamide,doxorubicin • L-Asparaginase • Midostaurin • Azacytidine • Decitabina	 1 (3) 1 (3) 13 (44) 1 (3) 4 (13) 4 (13) 3 (10) 5 (17) 4 (13)

Abbreviation: AML: Acute Myeloid Leukemia; ALL Acute Lymphoid Leukemia; COPD: Chronic obstructive pulmonary disease; DM: Diabetes Mellitus; FLAG: fludarabine, cytarabine, and granulocyte colony-stimulating factor; MDS: Myelodisplastic syndrome; NOS: Not otherwise specified; Ph: chromosome Philadelphia; PV; Polycythemia Vera; TKI: tyrosine kinase inhibitor

Table 2. TEG parameters during all time-points of the study

Test, Mean ± SD	**T0 (n=40)**	**T1 (n=29)**	**T2 (n=14)**	**T3 (n=25)**	*p value*
R, min	9.3 ±2.9	8.6 ± 2.4	12.4 ±4.0	8.3± 1.3	T0vsT1 =ns T1vsT3=ns **T1vsT2<*0.05*** **T2vsT3<*0.05***
K, min	3.2± 2.8	4.7±3.9	7.8±5.7	2.5± 1.4	**T0vsT1<0.05** **T1vsT2<*0.05*** ***T1vsT3<0.05*** **T2vsT3<*0.05***
Alpha-angle, °	50.7±15.5	44.2±16.5	29.3±11.8	61.2±9.6	T0vsT1<0.05 ***T1vsT2<0.05*** **T1vsT3<0.05** **T2vsT3 <*0.05***
MA, mm	49.1 ±16.3	41.3±16.8	25.98±10.6	59.6 ± 13.2	T0vsT1=ns ***T1vsT2<0.05*** **T1vsT3<*0.05*** **T2vsT3<*0.05***
CI, mm	- 0.15± 2.16	-0.22 ± 2.2	-1.95 ± 3.12	0.69 ± 2.0	T0vsT1=ns; *T1vsT3=ns* **T1vsT2<*0.05*;** **T2vsT3<*0.05***
TG, mm/min	604±184	531± 181	467 ± 184	735 ±177	T0vsT1=ns; ***T1vsT2<0.05*** **T1vsT3<*0.05*;** **T2vsT3<*0.05***

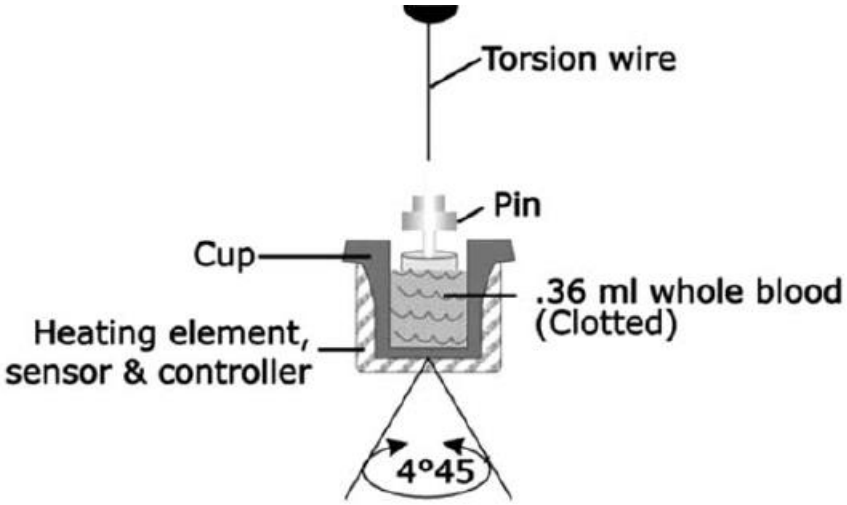
TEG: thromboelastography; R:reaction time; α (alpha-angle); K: kinetic time; MA: maximum amplitude; CI: coagulation index, TG: thrombin generation, ns: not significant

Table 3. Laboratory and conventional coagulation tests

Test, mean ± SD	T0 (n=40)	T1 (n=29)	T2 (n=14)	T3 (n=25)	*p value*
Hb (g/dl)	9.2 ±3.1	8.4 ± 0.9	8.3 ±0.5	9.6 ± 1.2	T0vsT1: <0.05; T1vsT2= ns; T1vsT3: <0.05; T2vsT3: <0.05
Hct (%)	27.1±5.1	25.0 ± 3.3	24 ±1.9	28 ± 4.0	T0vsT1: <0.05; T1vsT2= ns ;T1vsT3: <0.05; T2vsT3: <0.05
Wbc (x10^9/L)	31.0±48.0	5.0 ± 9.1	0.79 ±0.9	3.73 ± 2.3	T0vsT1: <0.05;T1vsT2: <0.05; T1vsT3: <0.05; T2vsT3: <0.05
Neu (x10^9/L)	4.4 ± 10.0	1.5 ± 2.9	0.11 ±0.2	2.01 ± 1.8	T0vsT1: <0.05; T1vsT2: <0.05; T1vsT3: <0.05; T2vsT3: <0.05
Lym (x10^9/L)	9.6 ±21.0	1.3 ± 1.8	0.47 ±0.3	1.24 ± 1.0	T0vsT1: <0.05; T1vsT2: <0.05; T1vsT3: <0.05; T2vsT3: <0.05
Mono (x10^9/L)	1.3 ± 22.0	2.6 ± 7.0	0.19 ±0.6	564 ± 565	T0vsT1: <0.05 ; T1vsT2:<0.05; T1vsT3: <0.05; T2vsT3: <0.05
Plt (x10^9/L)	66± 68	55±51	4.7 ± 4.0	136 ± 98	T0vsT1= ns; T1vsT2: <0.05; T1vsT3: <0.05; T2vsT3: <0.05
PT-ratio	1.1 ± 0.2	1.1 ± 0.1	1.17 ±0.3	1.12 ± 0.1	T0vsT1= ns; T1vsT2=ns; T1vsT3=ns; T2vsT3= ns
APTT, seconds	28 ± 5	29 ± 4	30 ±5	31.6 ± 5	T0vsT1=ns; T1vsT2=ns; T1vsT3=ns; T2vsT3= ns
FBG (mg/dl)	420 ± 195	335 ± 199	509 ±199	359 ± 208	T0vsT1: ns; T1vsT2: ns; T1vsT3: ns; T2vsT3: ns
DD (µg/L)	3827 ± 6174	2125 ±1.968	1469 ±1200	984 ± 1289	T0vsT1: ns; T1vsT2: ns; T1vsT3: <0.05; T2vsT3: <0.05

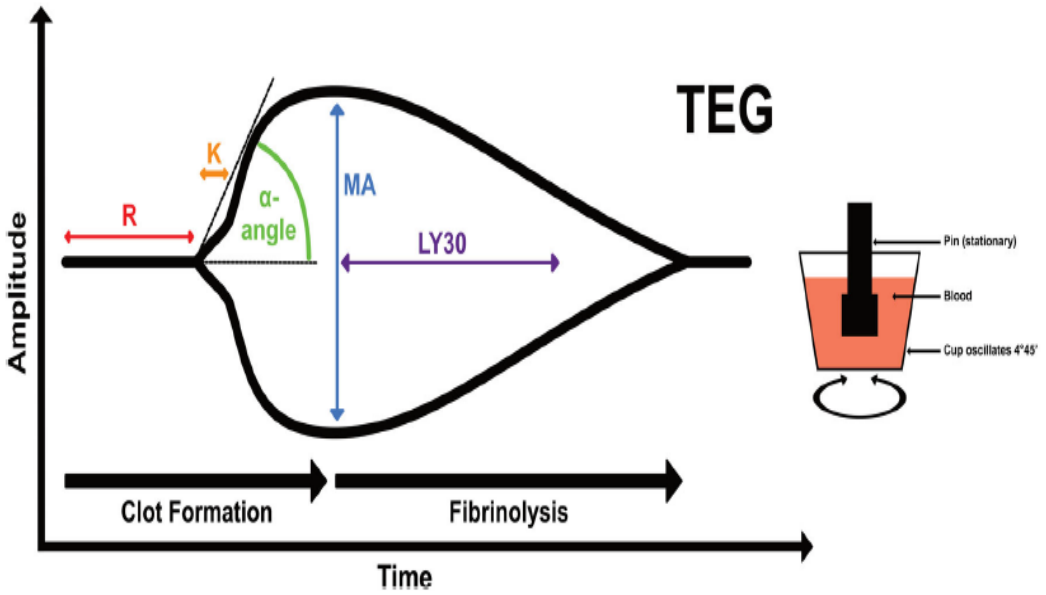
Abbrevations: Hb: hemoglobin, WBC: white blood cell count; Neu: neutrophils; Lym: lymphocytes; Mono: monocytes; Plt: platelets; PT:prothrombin time; APTT :activated partial thromboplastin time; DD:D-dimer; FBG: fibrinogen; NS: not significant

Figure 1



A depiction of a TEG device in which a pin suspended from a torsion wire is immersed in a cup of whole blood. The cup is held in a heating block and continually oscillates through 4_ 450 every 5 sec. Changes in viscoelastic clot strength are directly transmitted to the torsion wire and detected by an electromechanical transducer. (Adapted from TEG and ROTEM: technology and clinical applications Am J Hematol . 2014 Feb;89(2):228-32.)

Figure 2

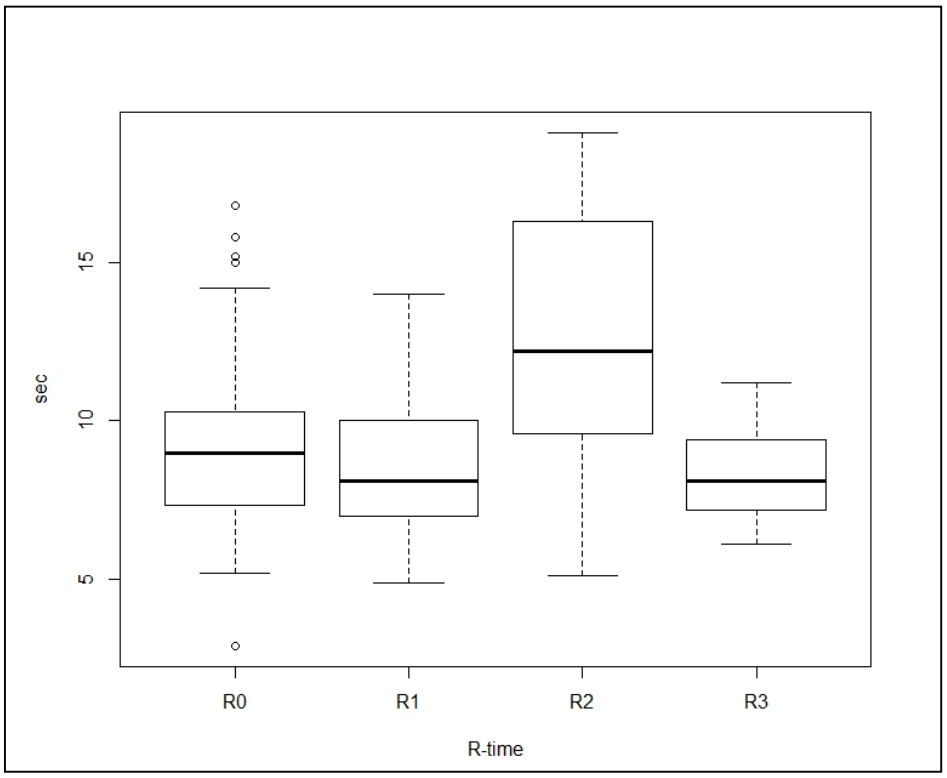


Depiction of a TEG. Reaction time (R) is the clotting time to cause a 2-mmdisplacement during initial clot formation. Kinetics (K) is the clot formation time. α-Angle is the rate of clot formation. Maximum amplitude (MA) is the maximumclot strength. Lysis at 30 minutes (LY30) is the percent decrease in amplitude 30minutes after achieving MA. As illustrated on the right-hand side, citrated blood is placed in a warmed cup into which a pin descends. As the cup rotates and the clot forms, the connection between the rotating cup and the pin induces torqueonthe pin,which is registeredby a transducer as a characteristic shovel-shaped curve.Abbreviations: TEG, thromboelastography (Adapted from Walsh et al. Semin Thromb Hemost 2019;45:354–372.)

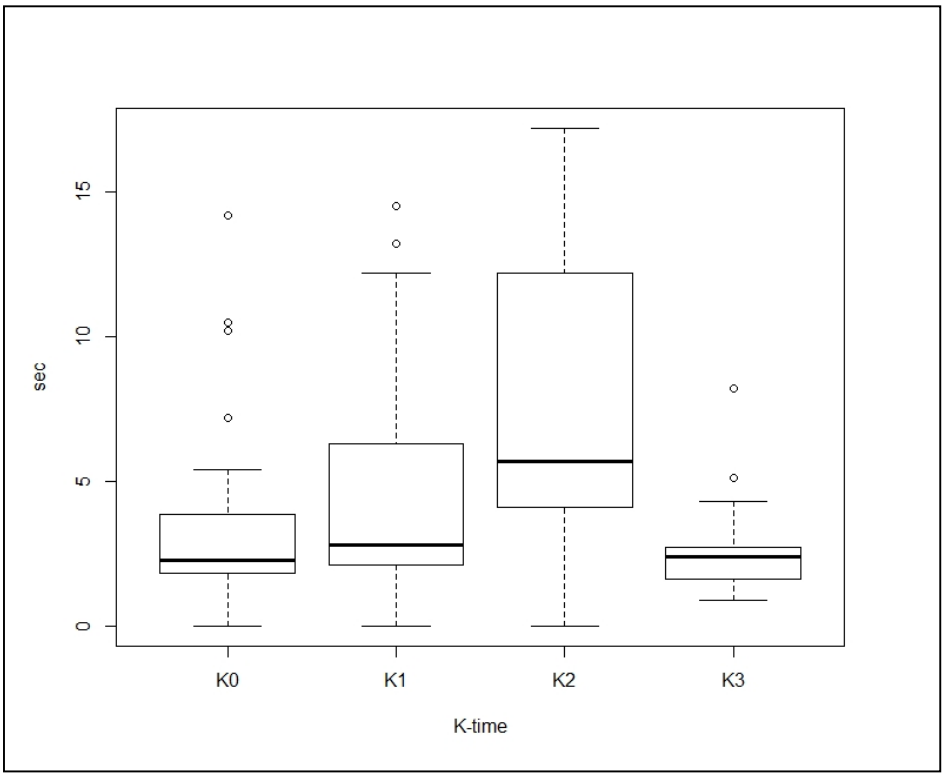
Figure 3

Time course of TEG-parameters in patient with acute leukemia during the course of disease and treatment

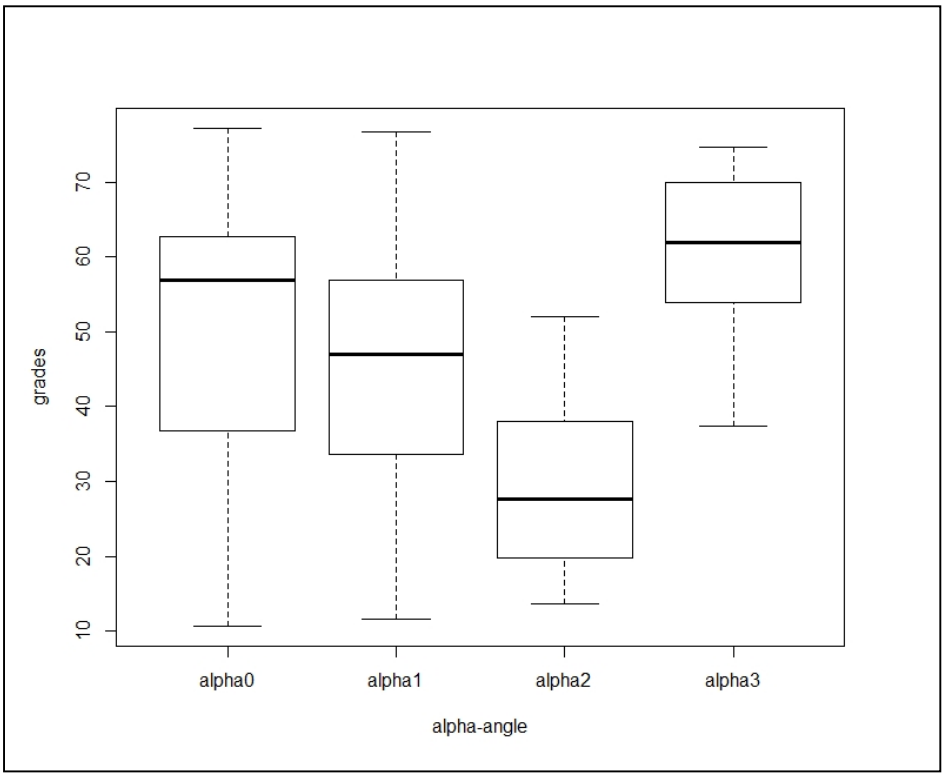
a) R-time at the 4 time-points previous by the study: T0 (diagnosis), T1 (during chemotherapy), T2 (during myelosuppression), T3 (after chemotherapy).



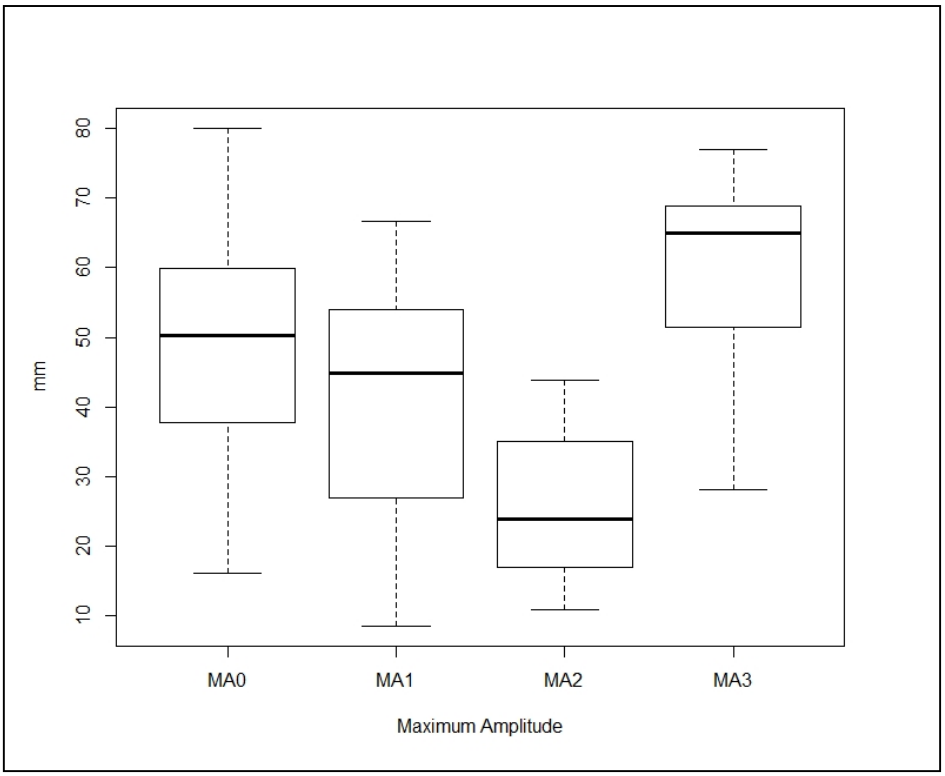
b) K-time at the 4 time-points previous by the study: T0 (diagnosis), T1 (during chemotherapy), T2 (during myelosuppression), T3 (after chemotherapy).



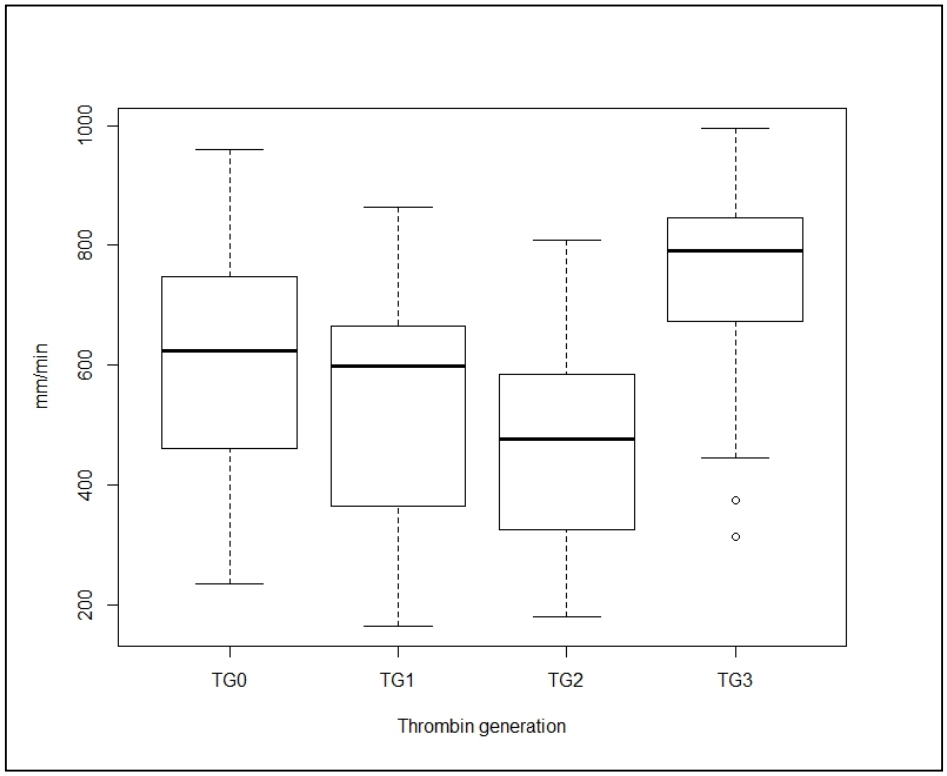
c) Alpha-angle at the 4 time-points previous by the study: T0 (diagnosis), T1 (during chemotherapy), T2 (during myelosuppression), T3 (after chemotherapy).



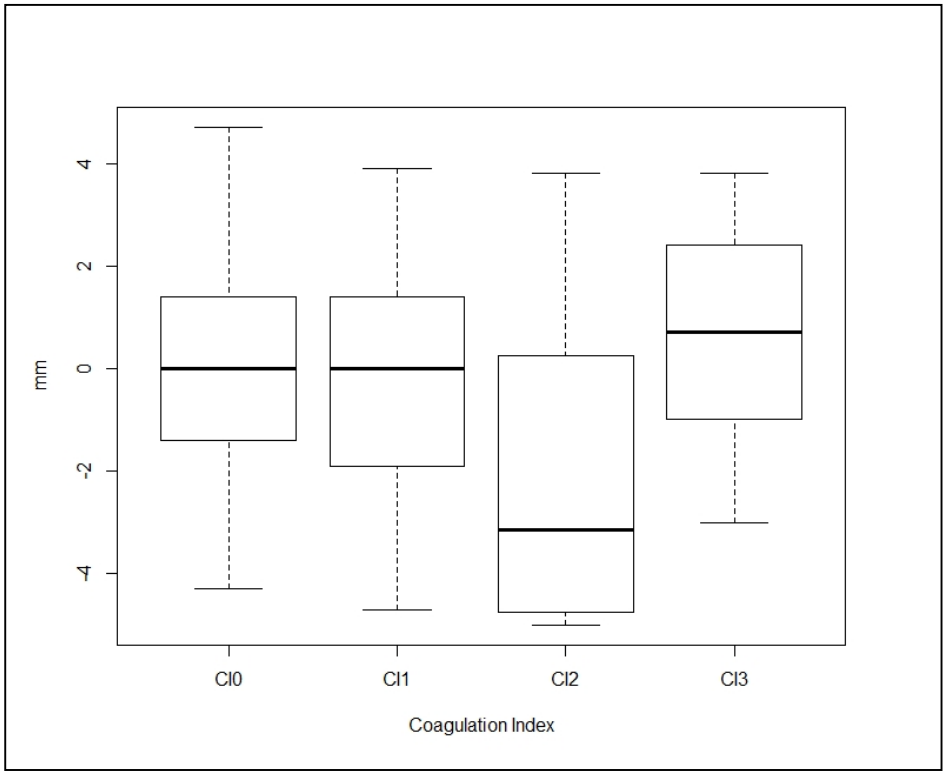
d) Maximum-amplitude at the 4 time-points previous by the study: T0 (diagnosis), T1 (during chemotherapy), T2 (during myelosuppression), T3 (after chemotherapy).



e) Thrombin generation at the 4 time-points previous by the study: T0 (diagnosis), T1 (during chemotherapy), T2 (during myelosuppression), T3 (after chemotherapy).



f) Coagulation index (CI) at the 4 time-points previous by the study: T0 (diagnosis), T1 (during chemotherapy), T2 (during myelosuppression), T3 (after chemotherapy).



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