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ORIGINAL ARTICLE

Global coagulation assays detect an early prothrombotic state in children with acute lymphoblastic leukemia

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Abstract

Background: Pediatric patients with acute lymphoblastic leukemia (ALL) are at highest risk of venous thromboembolism during the induction therapy (IT). These events are not predictable by conventional coagulation assays.

Objectives: To investigate the utility of global coagulation assays (GCAs) for assessing the hemostatic state in children with ALL during IT.

Methods: We included children with ALL (n = 15) and healthy controls (n = 15). Analyses were performed at different time points during IT of the AIEOP-BFM protocols. In addition to prothrombotic biomarkers, natural anticoagulant proteins, and *in vivo* thrombin generation (TG) markers, *ex vivo* TG was measured using the gold standard calibrated automated thrombogram method, automated ST Genesia, and thrombodynamics analyzer (TD). The latter also provided measurement of fibrin clot formation.

Results: Different from conventional coagulation assays and *in vivo* TG markers, *ex vivo* GCAs detected increasing prothrombotic changes during IT. Particularly, TG measured with TD as expressed by endogenous thrombin potential was already significantly elevated at days 8 to 12 ($P < .01$) and continued to increase during IT compared with prior to beginning treatment, indicating a very early shift toward a procoagulant state. A similar pattern was observed for the rate of fibrin clot formation (stationary rate of clot growth: $P < .01$ at days 8-12). Remarkably, in patients developing thrombotic complications (n = 5), both GCAs, ST Genesia and TD, showed a significantly higher endogenous thrombin potential very early (already at days 8-12, $P < .05$), well before clinical manifestation.

Conclusion: GCAs capture prothrombotic changes early during IT in ALL pediatric patients. If confirmed, this approach will allow tailoring thromboprophylaxis in children with ALL at highest risk for venous thromboembolism.

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KEYWORDS
acute lymphoblastic leukemia, fibrin clot formation, global coagulation assays, pediatric VTE, thrombin generation

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Children with ALL are at highest risk for venous thromboembolism (VTE), mainly during the induction phase of chemotherapy, with a reported incidence of up to 37%, which leads to significant morbidity [1–7]. The pathogenesis of thrombosis in patients with ALL is multifactorial and includes the disease itself, the administration of drugs, such as asparaginase and steroids, the presence of indwelling central venous line (CVL), and septic complications [7–10]. Particularly, acquired antithrombin (AT) deficiency secondary to asparaginase-induced asparagine depletion is considered one of the main mechanisms for the development of thromboembolic events during induction therapy (IT) [3–6]. On the other hand, these patients may be at increased risk of hemorrhagic complications due to, eg, intermittent low platelet counts and the need for multiple invasive procedures. Given this fragile hemostatic balance, primary thromboprophylaxis is currently neither common practice nor recommended in pediatric patients with ALL. Indeed, the randomized PREVAPIX-ALL (APIXaban compared with standard of care for PREVention of venous thrombosis in pediatric Acute Lymphoblastic Leukemia [ALL]) trial showed no significant decrease in VTE in pediatric patients with ALL who received thromboprophylaxis in a clinical context [11]. Therefore, identifying pediatric ALL patients at highest risk of VTE, who could benefit from thromboprophylaxis, is of relevant importance [1,9,11–13]. In pediatric ALL patients, routine coagulation tests are often altered, suggesting a bleeding tendency, while on the contrary, VTE is the greater risk. Current available *in vitro* routine laboratory tests are poor predictors of the overall hemostasis balance, especially in presence of such multiple hemostatic changes occurring in patients with ALL [8,14]. Indeed, prothrombin time (PT) and activated partial thromboplastin time (APTT) only partially explore and assess the coagulation process, as the end point of these tests (initial fibrin formation) occurs when only a tiny amount of thrombin (about 5%) is formed, leaving the remaining part of the clotting process undetected [15–17]. Moreover, PT and APTT are insensitive to natural anticoagulant factors deficiency, such as congenital protein C (PC), protein S (PS), and AT deficiencies [18,19].

To avoid these limitations, the use of global coagulation assays (GCAs) might represent a more comprehensive alternative approach [20–22]. The calibrated automated thrombogram (CAT) method, developed by Hemker et al. [23], is currently the gold standard for the measurement of thrombin generation (TG) and global assessment of coagulation potential [22]. However, it seems difficult to apply this method to clinical practice as it requires manual handling, shows high variability, lacks standardization [18], has unclear reliability to predict thrombotic risk [22], and needs further clinical validation [20].

In recent years, efforts have been made by manufacturers to improve existing methods or to introduce new GCAs. For instance, the fully automated benchtop analyzer ST Genesia (STG, Stago), now available to clinical laboratories, allows quantitative automated and standardized measurements of TG [24]. However, this tool does not explore fibrin clot formation (FCF). Thrombodynamics analyzer (TD) is a conceptually novel technique that allows the analysis of the spatiotemporal propagation of tissue factor (TF)-dependent and -independent coagulation [25–27]. Moreover, this technique not only enables the measurement of TG but also the analysis of FCF, thus adding a new important element in the assessment of the coagulation state.

Chemotherapy for children with ALL, according to Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP)-Berlin-Frankfurt-Münster (BFM) protocol [28,29], includes an IT with 4 drugs during the first 33 days, starting with daily steroids, weekly vincristine, and daunorubicin from day 8 for a total of 4 doses and 2 doses of pegylated (PEG)-asparaginase at day 12 and day 26, respectively. The induction phase is followed by the first part of the consolidation phase starting at day 36 (detailed in Supplementary Figure S1). Here, we studied the coagulation potential in children with ALL during the induction phase of chemotherapy, in which the risk of VTE is at the highest, and performed a follow-up at day 50. We employed the gold standard technique (CAT) and new-generation global assays, such as STG and TD. Our aim was to identify whether the parameters of TG and/or FCF could be used to describe subtle changes in the hemostatic status of this patient population and to potentially identify ALL patients at highest risk for VTE.

2 | METHODS

2.1 | Study design, patient groups, and healthy controls

This is a pilot observational study aimed at exploring the hemostatic balance in patients with ALL during chemotherapy. Of note, at our institution, we do not perform routine thromboprophylaxis in children with ALL, and no patient enrolled in this study received it. Fifteen children under the age of 18 years with ALL diagnosed and treated according to the AIEOP-BFM protocol (2009/2017) [28,29] at the Pediatric Hematology/Oncology Unit, Division of Pediatrics, Lausanne University Hospital (CHUV), Lausanne, Switzerland, were prospectively enrolled in the study from August 25, 2020, to March 22, 2022. In addition, 15 healthy controls (aged <19 years) were recruited at the Pediatric Hematology/Oncology Unit, Division of Pediatrics, Lausanne University Hospital (CHUV), among patients with a past history of

cancer in complete remission at a median follow-up time (since end of treatment) of 3.2 years (IQR, 2.2-4.8). This study was approved by the local independent ethics committee (CER-VD 2020-00742) and conducted according to the Declaration of Helsinki. All participants/representatives gave their written informed consent prior to their participation.

2.2 | Blood collection and plasma preparation

Blood was collected in patients with ALL at different time points (detailed in Supplementary Figure S1) before and during IT, namely at day 0 (baseline, within 24 hours prior to start of treatment), between day 8 and 12 (under steroids treatment and before the first dose of PEG-asparaginase), day 22 (10 days following the first dose of PEG-asparaginase), day 33 (end of IT), and day 50 (prior to third dose of PEG-asparaginase). In all children with ALL, blood was taken from the CVL. At our institution, a CVL (either a peripherally inserted central catheter [PICC] or a nontunneled CVL) is usually placed in the upper venous system at the time of starting treatment. Thereafter, at the end of induction and prior to starting the consolidation phase on day 36, a tunneled CVL (such as a port-a-cath) is then inserted. Before insertion of the tunneled CVL, a Doppler ultrasound (DUS) is routinely performed to evaluate patency of the upper venous system. In this cohort, 13 patients had PICC, and 2 patients had a nontunneled CVL placed at a median time of −0.5 days (IQR, −1 to 0) of treatment start. In all included children with ALL, the port-a-cath was inserted at median time of 28 days (IQR, 28-32) of treatment start. In addition, a single blood collection was obtained from 15 healthy controls. In this case, blood was withdrawn from the antecubital vein using a 20- to 25-gauge needle. Blood was collected in tubes containing 0.106 mol/L sodium citrate (S-Monovette, Sarstedt AG & Co KG). Platelet-poor plasma (PPP) was prepared by double centrifugation at 1800 × *g* for 10 minutes at room temperature. Platelet-free plasma was prepared with a first centrifugation at 1600 × *g* for 15 minutes, followed by a second centrifugation at 1600 × *g* for 20 minutes at room temperature. Plasma samples were divided into aliquots, snap-frozen, stored at −80 °C, and subsequently used to perform analysis.

2.3 | VTE diagnosis and imaging modalities

The diagnosis of VTE was based on clinical suspicion and had to be confirmed by 1 or more suitable imaging methods (eg, DUS, magnetic resonance imaging, and/or computerized tomography scan). In addition, at our center, DUS is routinely performed to evaluate patency of the upper venous system before inserting a tunneled CVL prior to consolidation phase. Specifically, DUS of the upper extremity venous system was performed in 14 out of 15 (93%) patients at median time of 28 days (IQR, 28-32) of treatment start. Finally, all CVLs are checked routinely for patency and/or dysfunction prior to their use by the nurse and at any time in case of symptoms. Intermittent dysfunction of the CVL by a clot at the tip of the catheter was not considered a thrombotic event as long as CVL patency was restored.

2.4 | Routine coagulation analyses and *in vivo* marker of TG

Routine coagulation tests PT, APTT, and fibrinogen; procoagulant biomarkers coagulometric factor (F)VIII assay (FVIII:C) and von Willebrand factor (VWF) activity (VWF:Ac); and natural anticoagulants AT, PC activity, and PS free antigen were measured on a Sysmex CS5100 coagulation analyzer (Siemens Healthineers) according to the manufacturer's instructions. As *in vivo* markers of coagulation, prothrombin fragment F1 + 2 (F1 + 2) and thrombin AT complex (TAT) were measured by a quantitative enzyme immunoassay according to the manufacturer's protocols (Enzygnost F1+2 micro and Enzygnost TAT micro, respectively, Siemens Healthineers), and D-dimer (DD) were measured by an automated quantitative immunoassay (INNOVANCE D-dimer, Siemens Healthinneers) according to the manufacturer's instructions.

2.5 | GCAs

2.5.1 | CAT

TF-triggered TG was measured by CAT analysis according to the method of Hemker et al. [30]. PPP was thawed for 10 minutes at 37 °C and then immediately analyzed. Measures were carried out in a 96-well transparent, round bottom plate, and the readings were done using a Fluoroskan Ascent (Thermo Fisher Scientific) plate reader at 37 °C with the wavelength set at 390 nm (excitation) and 460 nm (emission). For each patient, 20 μL of prewarmed trigger solution (PPP-Reagent, Stago) containing final concentrations of 5 pM TF and 4 μM phospholipids and 20 μL of prewarmed calibrator (Thrombin Calibrator, Stago) were added to different wells in triplicate. Finally, 80 μL of plasma was added to each well. The required amount of the thrombin specific fluorogenic substrate Z-Gly-Gly-Arg-AMC (7-amino-4-methylcoumarin; Fluo-Substrate, Stago) was added to a prewarmed buffer containing calcium (Fluo-Buffer, Stago) in a ratio of 1:40; then, 20 μL of this mixture (Fluo-Substrate and Fluo-Buffer = FluCa) were dispensed to each well immediately prior to the beginning of the measurement. Measurements were performed in triplicate for each experiment. TG was calculated by the Thrombinoscope software (version 5.0.0.742, Thrombinoscope).

2.5.2 | STG

Analyses were performed according to the manufacturer's instructions on thawed citrated PPP (10 minutes at 37 °C) on the fully automated STG analyzer (Stago) using the STG-ThromboScreen Kit (Stago). A solution containing the fluorogenic substrate

Z-Gly-Gly-Arg-AMC together with calcium chloride (STG-FluoStart, Stago) was added to a known amount of thrombin (STG-Thrombical, Stago) to perform a calibration curve once a day prior to the start of the measurement in plasma. A solution containing a fixed concentration of AMC (STG-FluoSet, Stago) was incubated with the STG-Thrombical; this allows for adjustment of the calibration curve for the optical features of the plasma sample color. Coagulation was triggered in presence of phospholipids and an intermediate concentration of TF (STG-ThromboScreen, Stago) in presence or in absence of thrombomodulin (TM) after the automatic addition of the fluorogenic substrate and calcium (STG-FluoStart, Stago). Fluorescence was measured until completion of the TG curve. Analyses were performed in duplicate at 37 °C without and with TM using a wavelength of 377 nm (excitation) and 450 nm (emission) for the fluorescence reading.

2.5.3 | Thrombodynamics assay

TD analyzer, an optical-based video microscopy method, was used to monitor TG and FCF simultaneously. The assay was done using the PLS Kit (HemaCore LLC) according to the instructions of the manufacturer in thawed citrated platelet-free plasma. Briefly, 120 µL of plasma was added to Reagent I containing lyophilized corn trypsin inhibitor (CTI) to prevent the activation of the contact activation pathway, a lyophilized fluorogenic substrate (Z-Gly-Gly-Arg-AMC), and 5 µL of Reagent PLS containing phospholipids vesicles (final concentration 4 µM). After incubation at 37 °C for 15 minutes, the sample was recalcified (Reagent II) and transferred to the experimental cuvette. Coagulation was triggered by the addition to the cuvette of a plastic insert coated with immobilized TF (100 pmol/m^2). The addition of CTI in this assay is needed since there is no TF in the plasma at distance from the activator. Images of clot growth were recorded by the analyzer, measuring the increase of the light scattering in the area of clot formation, and specific quantitative parameters describing the spatiotemporal dynamics of TG and FCF were calculated by the TD software (version 4.1.3, HemaCore LLC). Since TD is an optical system, samples that were too lipemic after thawing (2 out of 15) were centrifugated at 14 000 × *g* for 10 minutes in order to be measured.

2.5.4 | TG parameters

The following parameters were measured for TG: lag time (LT, minutes), the time needed to initiate TG; peak height (PH, nM), the maximal concentration of thrombin produced; the velocity index (VI, nM/min), the maximum speed of thrombin production; the endogenous thrombin potential (ETP, nM * minutes), the total amount of thrombin formed [23]; the percentage of ETP inhibition, measured after the addition of TM (calculated by the following formula: $ETP^{+TM} - ETP^{-TM}/ETP^{+TM}$). The percentage of ETP inhibition reflects the activity of PC. CTI was not used for CAT and STG assays because the TF concentration was >1 pM [31].

2.5.5 | FCF parameters

The following parameters were measured: initial rate of clot growth (Vi, mm/min) and stationary rate of clot growth (V, µm/min), reflecting the speed at which fibrin is produced in the initial (TF-dependent) and amplification (TF-independent) phases of coagulation respectively; the fibrin clot size (CS, µm), measured at 30 minutes from the initiation of the coagulation; and the spontaneous clot formation time (Tsp, minutes), indicating high hypercoagulant plasma.

Of note, for *ex vivo* TG, a procoagulant tendency will be indicated by a significant increase in the amount of thrombin formed (ETP and PH, respectively) and in the rate of thrombin formation (velocity index), as well as by a significant decrease of LT and TM-mediated ETP inhibition (highlighting an impairment of the PC pathway). Similarly, for *ex vivo* FCF, a procoagulant tendency will be indicated by a significant increase in the clot formation rate and size (Vi, V, and CS, respectively) and by a faster appearance of spontaneous clots (Tsp).

2.6 | Statistical analyses

Statistical analysis was performed with GraphPad Prism 9, v9.5.1 (GraphPad Software).

The evolution of the hemostatic state in patients during the IT was studied by comparing the median values of the TG and FCF parameters measured in the cohort at days 8 to 12, day 22, day 33, and day 50 to the values measured at day 0. This was done by a nonparametric, nonmatching Kruskal–Wallis test or parametric 1-way analysis of variance according to data distribution. Data on day 50 from patients who received anticoagulation were excluded from the analysis. In addition, a nonparametric *t*-test (Mann–Whitney U-test) or unpaired *t*-test was performed to compare the patients before the onset of the treatment (day 0) or prior to third dose of PEG-asparaginase (day 50) with healthy controls according to data distribution. For 2 patients, the sample at day 0 was not available, so they were excluded from this comparison. *P* value significance was set at <.05.

3 | RESULTS

3.1 | Clinical and laboratory characteristics of patients and controls

Baseline clinical and hematologic characteristics of the included ALL patients and healthy pediatric controls are provided in the Table.

3.2 | *In vivo* markers of TG

Patients' coagulation profile prior to treatment (day 0) was different from control group since patients had higher TG *in vivo* biomarkers (Figure 1 and Supplementary Table S1). However, in the studied

TABLE Baseline clinical and hematologic characteristics of patients with acute lymphoblastic leukemia and healthy controls.

Variables	ALL[a] (*n* = 15)	Healthy controls (*n* = 15)	*P* value
Age, y	6.6 (3.6-8.2)	10.2 (6.7-11.4)	<.05
Female, *n* (%)	8 (53)	6 (40)	NS
ALL phenotype B, *n* (%)	14 (93)	NA	NA
VTE cases, *n* (%)	5 (33)	NA	NA
Hemoglobin, g/L	101 (86-116)	124 (119-134)	<.001
Platelet count ($\times 10^9$/L)	92 (65-149)	292 (216-337)	<.001
WBC count ($\times 10^9$/L)	4.8 (3.9-9.4)	6.7 (6.0-9.5)	NS
Neutrophils ($\times 10^9$/L)	0.7 (0.4-2.8)	3.5 (2.4-5.0)	<.01
Monocytes ($\times 10^9$/L)	0.0 (0.0-0.3)	0.7 (0.5-0.7)	<.01
PT, % s	80 (73-95) 11.7 (12.1-11.1)	90 (80-100) 11.5 (12.1-10.7)	NS
APTT, s	34 (29-43)	29 (28-33)	NS

Values are given as median and interquartile range (25th-75th quartiles) or number and percentages. Comparison of patients with ALL vs healthy controls was performed with Mann–Whitney U-test, unpaired *t*-tests, or Fisher's exact test as appropriate.

ALL, acute lymphoblastic leukemia; APTT, activated partial thromboplastin time; NA, not applicable; NS, $P > .05$; PT, prothrombin time; VTE, venous thromboembolism; WBC, white blood cell count.

[a] Values at day 0 (maximum 24 hours before start of treatment).

population, F1 + 2 and TAT (Figure 1A, B), reflecting *in vivo* TG, did not show an increased procoagulant state during treatment. Noteworthy, DD (Figure 1C), as an indicator of fibrin degradation, significantly decreased during treatment.

3.3 | *Ex vivo* TG

Among the *ex vivo* TG parameters measured (Figure 2 and Supplementary Table S2), the ETP (an indicator of the overall TG potential) and the LT (the time necessary for the first amounts of thrombin to be generated) detected very early during IT, a shift toward a procoagulant state. Indeed, the TD assay revealed a significant ETP increase already at days 8 to 12 (median, 2370 nM*min; IQR, 1928-2565) compared with day 0 (median, 1612 nM*min; IQR, 1376-2013; $P < .01$; Figure 2A) and an LT shortening (median, 3.2 min; IQR, 2.9-3.6; $P < .01$) in comparison with day 0 (median, 3.7 min; IQR, 2.9-4.9; Figure 2E, F). Of note, the TG potential of patients assessed by TD on day 50 remained higher than that in controls.

3.4 | *Ex vivo* FCF

FCF was markedly accelerated and increased in ALL patients as the treatment progressed (Figure 3, Supplementary Table S2). Notably, as for TG, FCF indicated a change toward hypercoagulation already at days 8 to 12, as indicated by the increase of Vi (median, 79 μm/min; IQR, 74-84), V (median, 67 μm/min; IQR, 62-81), and CS (median, 2082 μm; IQR, 2022-2477) median values compared with day 0 (Vi, median, 72 μm/min; IQR, 70-77; $P < .05$; V, median, 51 μm/min; IQR, 45-55; $P < .01$; CS, median, 1762 μm; IQR, 1626-1905; $P < .01$). In addition, in patients with ALL, the Tsp, indicating the presence of hypercoagulable plasma, was shortened already at days 8 to 12. Of note, only the Vi and CS were increased in patients at day 0 compared with controls (Figure 3, Supplementary Table S2).

3.5 | Changes of pro- and anticoagulant proteins during IT

Changes in single proteins acting in the coagulation process as pro- or anticoagulant forces were also evaluated (Figure 4 and Supplementary Table S1).

The procoagulant parameters in patients with ALL at day 0 (FVIII:C, median, 165%; IQR, 120-210; VWF:Ac, median, 181 IU/dL;

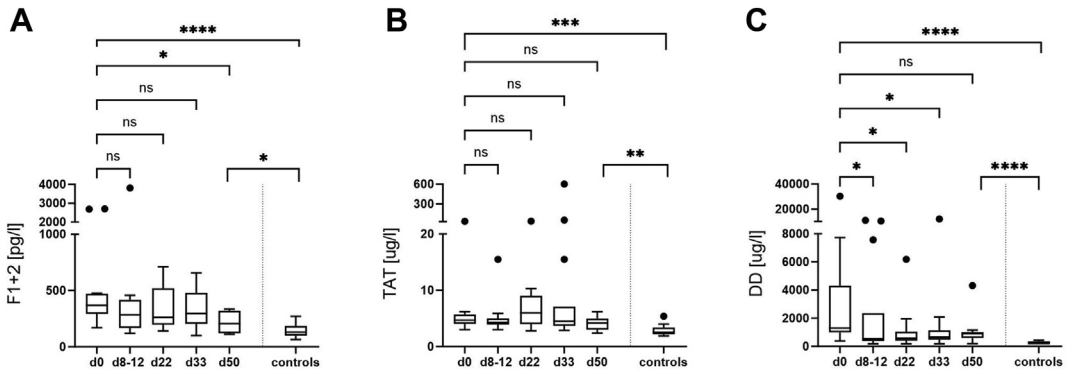

FIGURE 1 Time course of *in vivo* markers of thrombin generation in patients with acute lymphoblastic leukemia during the induction therapy. Prothrombin fragment F1 + 2 (F1 + 2; A), thrombin-antithrombin complex (TAT; B), and D-dimer (DD; C) were measured at day 0 (d0, baseline, within 24 hours prior to the start of the treatment) and at days 8 to 12 (d8-12), day 22 (d22), day 33 (d33), and day 50 (d50) of induction therapy. The box plots span from 25th to 75th quartiles (interquartile range, IQR), and the line inside the box indicates the median value. Whiskers are calculated using Tukey's method (lowest value close to the 25th percentile minus 1.5 IQR, and higher values close to the 75th percentile plus 1.5 IQR). Ns $P > .05$, *$P \leq .05$, **$P \leq .01$, ***$P \leq .001$, and ****$P \leq .0001$.

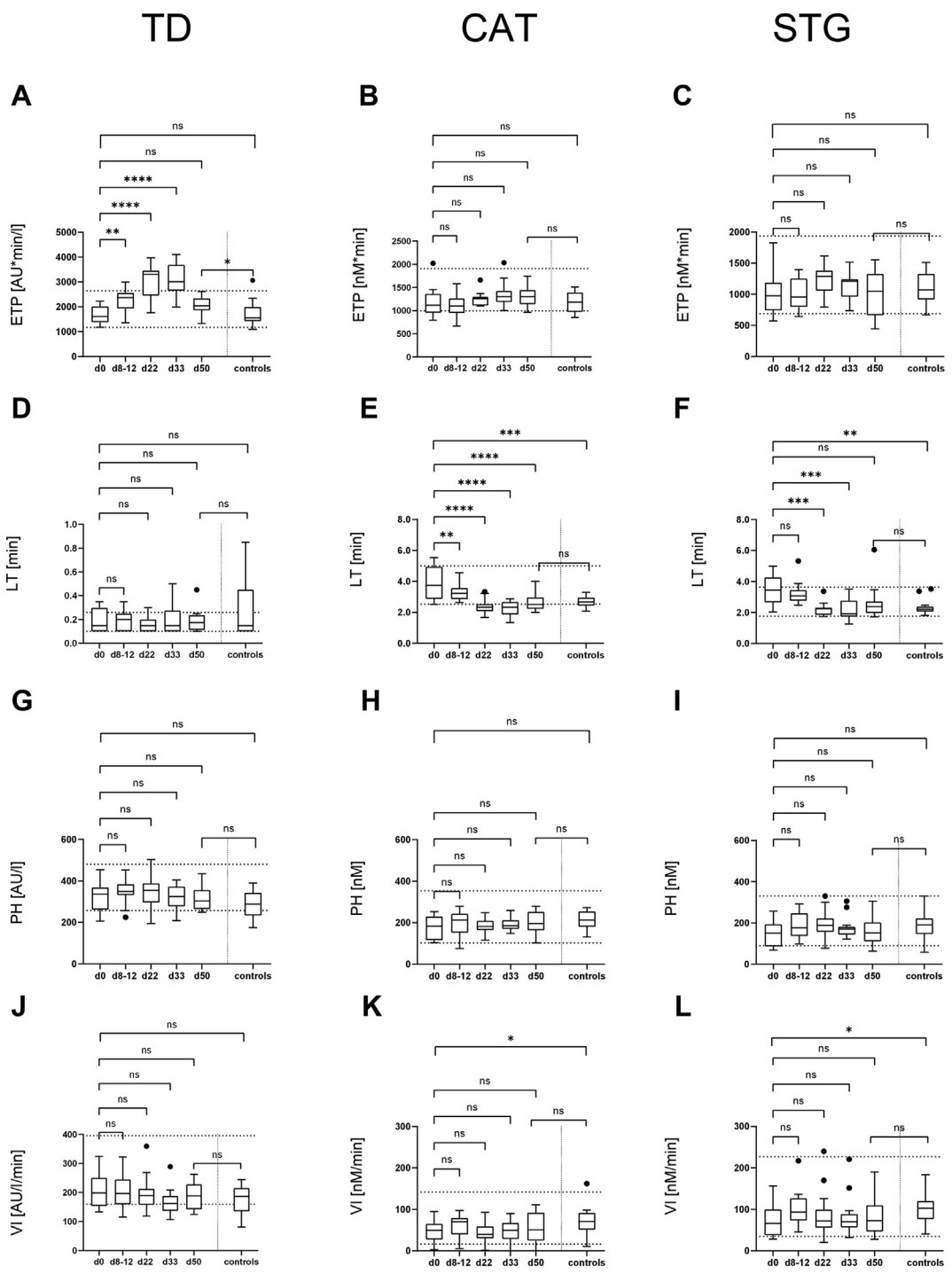

FIGURE 2 Time course of thrombin generation in patients with acute lymphoblastic leukemia during the induction therapy and controls. *Ex vivo* thrombin generation parameters (endogenous thrombin potential [ETP], lag time [LT], thrombin peak height [PH], and velocity index [VI]) were measured on 3 different devices. (A–J) Thrombodynamics analyzer, (B–K) calibrated automated thrombogram, and (C–L) ST Genesia at 5 time points: day 0 (d0), days 8 to 12 (d8-12), day 22 (d22), day 33 (d33), and day 50 (d50). The box plots span from 25th to 75th quartiles (interquartile range, IQR), and the line inside the box indicates the median value. Whiskers are calculated using Tukey's method (lowest value close to the 25th percentile minus 1.5 IQR, and higher values close to the 75th percentile plus 1.5 IQR). Ns $P > .05$, $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$.

IQR, 147-269; fibrinogen, median, 3.6 g/L; IQR, 2.6-4.6) were higher than in controls (FVIII:C, median, 107%; IQR, 97-126; $P < .01$; VWF:Ac, median, 111 IU/dL; IQR, 92-175; $P < .01$; fibrinogen, median, 2.6 g/L; IQR, 2.3-2.9; $P < .05$; Figure 4A–C). However, FVIII:C and VWF:Ac did not significantly change during IT (Figure 4A, B). Differently, fibrinogen levels significantly decreased early and throughout

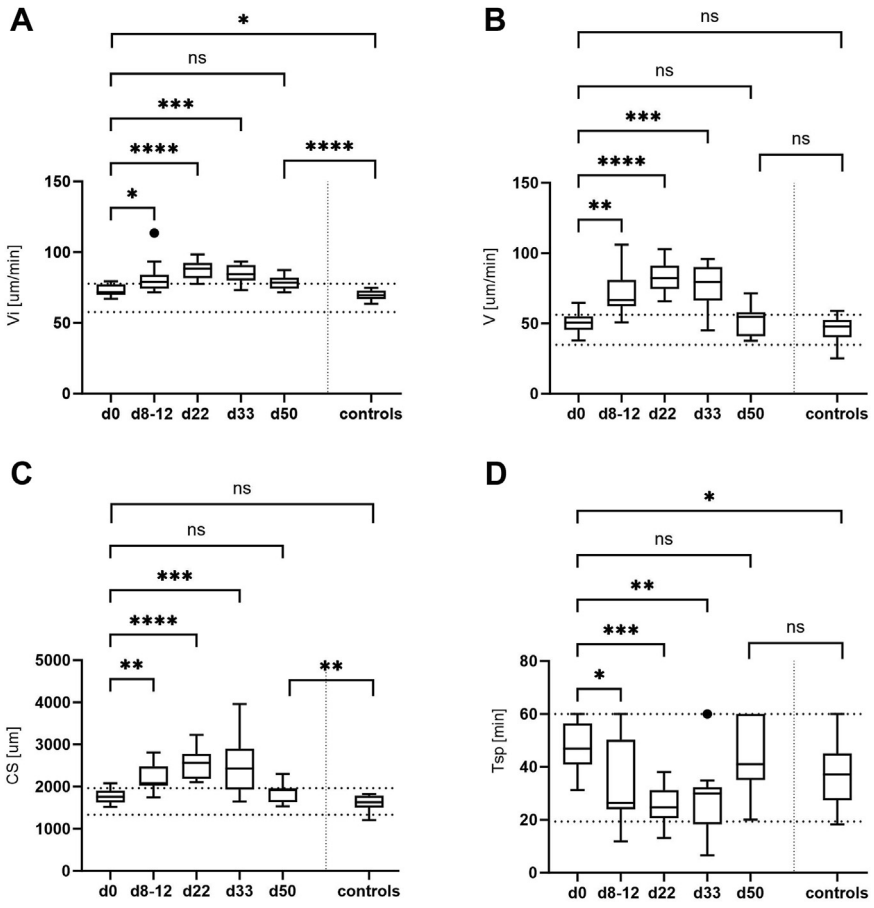

FIGURE 3 Time course of fibrin clot formation parameters in patients with acute lymphoblastic leukemia during the induction therapy and controls. (A) Initial rate of clot formation (Vi), (B) stationary rate of clot formation (V), (C) clot size (CS), and (D) time to spontaneous clotting (Tsp) were measured at day 0 (d0) and at days 8 to 12 (d8-12), day 22 (d22), day 33 (d33), and day 50 (d50) of the induction therapy. Data are median values and IQRs. Ns $P > .05$, $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$.

the treatment (lowest at day 22 with a median of 0.5 g/L; IQR, 0.5-0.7; $P < .0001$; Figure 4C).

Natural anticoagulant proteins AT, PC, and PS did not differ between patients at day 0 and controls. Most interestingly, they significantly increased with a peak at days 8 to 12 of treatment and decreased thereafter until day 33 (Figure 4D–F). In line with the trend observed for PC and PS, the median value of ETP inhibition (by the addition of TM in the STG assay) was slightly increased at days 8 to 12 and then decreased in comparison with days 8 to 12 after PEG-asparaginase treatment (Supplementary Figure S2).

3.6 | Incidence of VTE

Five (33%) out of 15 children with ALL developed a VTE at a median time of 31 days (IQR, 28-58). In 3 (60%) out of 5 patients, VTE was catheter-related (PICC line) and affected the superficial and deep vein system of the upper extremity. In 1 out of 3 patients, catheter-related VTE was symptomatic with arm swelling. Two other children had cerebral VTE diagnosed by magnetic resonance imaging. One case presented with focal seizures with thrombosis of the right posterior parietal cortical vein of the parasagittal veins with extension into the superior longitudinal sinus. The second case was asymptomatic with a subacute thrombosis of the left superior anastomotic (Trolard) vein.

3.7 | Early changes of the coagulation potential in patients with VTE

We compared the coagulation potential in the group of patients who had VTE with those who did not (NoVTE; Figure 5, Supplementary Table S3). On day 0, ETP median values in the 2 groups were already similar on days 8 to 12; the VTE group had higher ETP values than the NoVTE group. Specifically, on days 8 to 12, the unpaired mean difference between VTE and NoVTE was 313 (95% CI, 80-548) and 490 nM*min (95% CI, 72-908) based on the STG and TD assays, respectively (Figure 5, Supplementary Table S3). Of note, except for DD, which was higher in the NoVTE group, no other coagulation test, including routine coagulation parameters (TP and APTT), *in vivo* TG markers (F1 + 2 and TAT), and/or pro- or anticoagulants factors (fibrinogen, FVIII:C, VWF:Ac, AT, PC, and PS) differed between the NoVTE and VTE groups (data not shown).

4 | DISCUSSION

Increased hemostatic activity and thrombotic complications are frequently observed in patients with cancer and, particularly, in children with ALL. However, primary thromboprophylaxis in this population, also considering the potential associated risks, does not

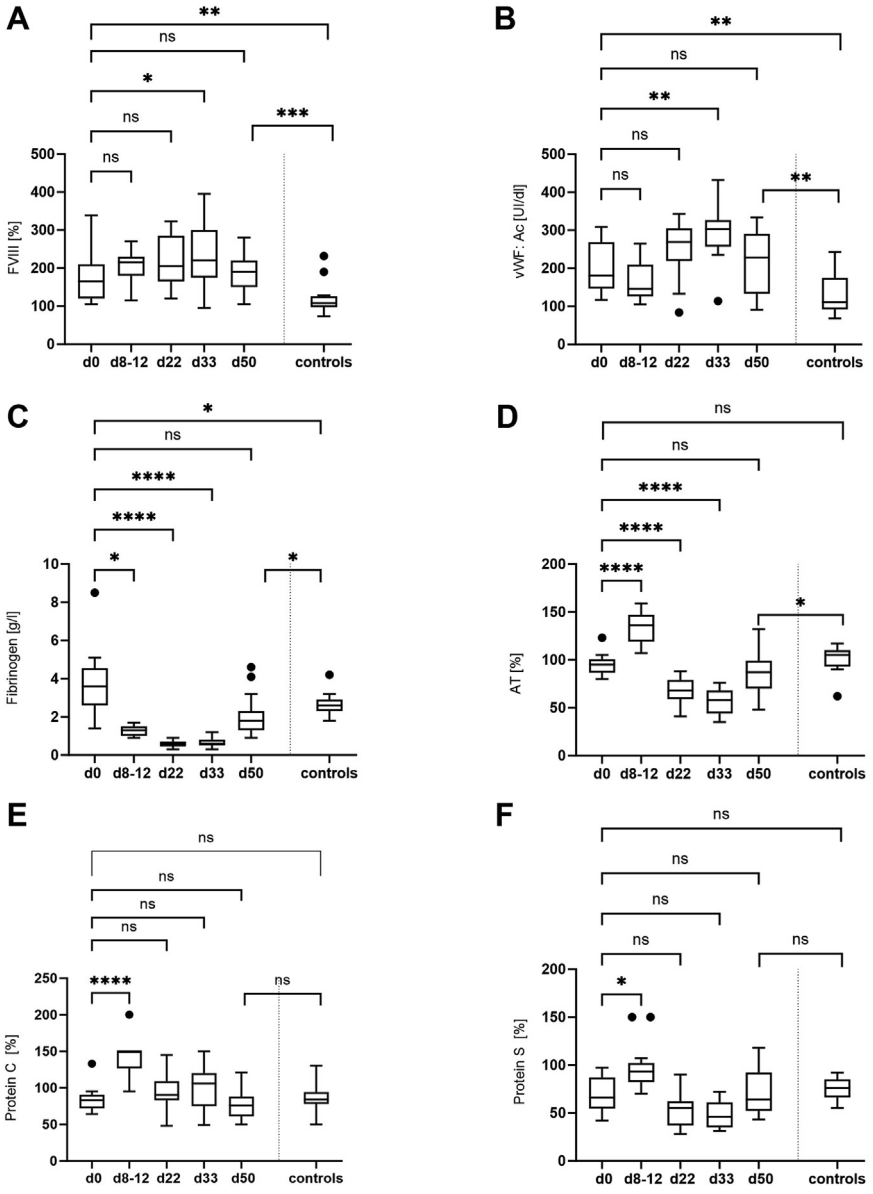

FIGURE 4 Time course of various coagulation parameters during the induction therapy in patients with acute lymphoblastic leukemia. (A) Coagulation factor VIII activity (FVIII:C, %), (B) von Willebrand factor activity (VWF:Ac, IU/dL), (C) fibrinogen level (g/L), (D) antithrombin activity (AT, %), (E) protein C activity (%), and (F) protein S free antigen (%). Data are median values and IQRs. Ns $P > .05$, $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$. d, day.

currently represent the standard of care. Prediction of thromboembolic complications in pediatric patients with ALL remains an unmet need [1,9,11–13]. Emerging laboratory techniques may respond to this concern, and GCAs have been useful in many clinical situations [32]. However, few studies assessing the coagulation potential of children with ALL treated with steroids and asparaginase during IT are available [33,34]. Therefore, we characterized, using different GCAs, the coagulation profiles of 15 patients with ALL before and during IT according to the AIEOP-BFM protocol 2009/2017. The comparison of patients at day 0 with healthy controls showed that *in vivo* TG markers (F1 + 2 and TAT) were significantly higher in patients with ALL (Figure 1, Supplementary Table S1). However, these *in vivo* markers of TG were not able to reflect the change toward a procoagulant state during induction. These findings are consistent with previous studies documenting an increased TG at diagnosis before starting antileukemic therapies [3,35–38] and a lack of significant changes in TAT values during induction phase [35,36,39]. In addition, and as expected, compared with healthy controls, patients with ALL at day 0 had significantly lower levels of hemoglobin, as well as decreased numbers of platelets, neutrophils, and monocytes (Table), which may reflect a decreased production of the myeloid cell lineage secondary to the leukemic infiltration of the bone marrow.

4.1 | GCAs detect an early increase in TG

Antileukemic therapy for children with ALL is based on the use of asparaginase, an enzyme that impairs protein synthesis by depleting plasma asparagine and glutamine, on which the growth of leukemic cells relies. As consequence, during chemotherapy, the synthesis of

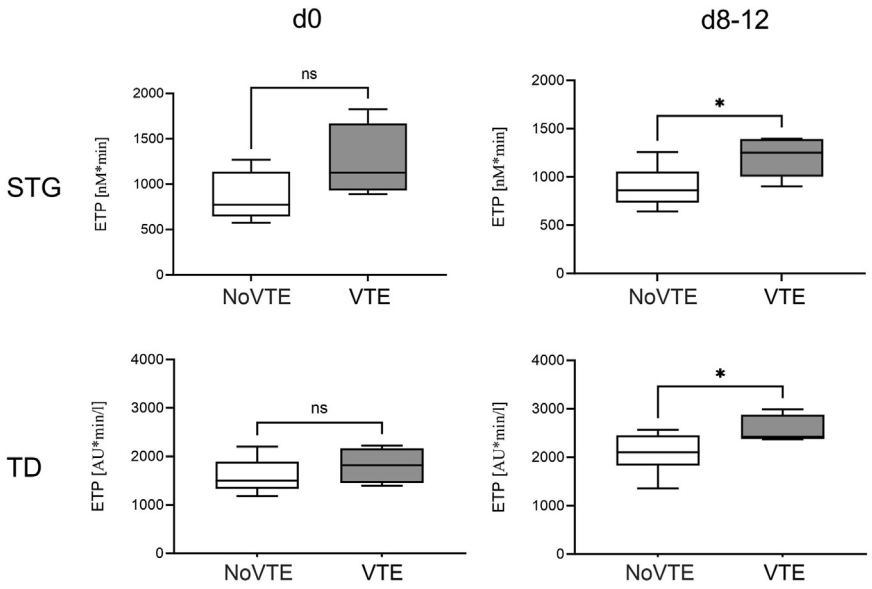

FIGURE 5 Early changes in the procoagulant state in patients with venous thromboembolism (VTE) compared with patients without VTE (NoVTE). Comparative boxplots for endogenous thrombin potential (ETP) measured at day 0 (d0) and days 8 to 12 (d8-12) with ST Genesia (STG) and thrombodynamics analyzer (TD) in patients who developed thrombotic complications (VTE, gray box) compared with NoVTE patients (white box). Data are median values and IQRs. Ns $P > .05$, $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$.

different hemostatic proteins (either pro- or anticoagulant) produced in the liver is affected [8,9]. Furthermore, asparaginase is usually given in combination with corticosteroids, which can also influence the coagulation system [9]. As also suggested by others [13,34], we hypothesized that GCAs are a promising approach to studying hemostasis when multiple changes in pro- and anticoagulant forces occur. Unlike routine coagulation tests that focus on specific pathways, GCAs represent a more comprehensive tool for evaluating hemostasis. Indeed, these assays reflect the complex interplay between procoagulant and anticoagulant elements, offering a global picture of the hemostatic balance [20–22,40]. For instance, TG can reveal both a PEG-asparaginase–induced decrease of AT (velocity index) and a decrease of PC/PS (TM-induced ETP inhibition) [41]. Differently from *in vivo* TG biomarkers, *ex vivo* TG measured by GCAs showed that the coagulation profile of ALL patients shifted very early during treatment toward an increased procoagulant state (Figures 2 and 3 and Supplementary Table S2). We observed differences among the 3 global assays used (CAT, STG, and TD) in their ability to capture changes in TG. In agreement with previous studies performed with CAT [13,34,42], we found an increased overall TG (ETP) also employing the TD method, documenting a procoagulant state in pediatric ALL patients during IT.

However, in our cohort, the CAT and the STG methods were not sensitive enough to show a significant difference in TG among the time points measured. We do not exclude that this inability of CAT and STG methods to capture differences in ETP compared with TD in this study may be due to the small sample size or to different experimental conditions, which hinder study comparison (eg, fresh [13] vs frozen plasma [34,39,42] or the use of TF at different concentrations; 1 pM [34,39,42] vs 5 pM [13]). We chose to work with a TF concentration of 5 pM with CAT and STG to be able to compare their results with TD, in which the coagulation is triggered by a surface coated with a TF density of 100 pM/m^2, which is considered equivalent to 5 pM [43]. On the other hand, the increased sensitivity of TD may have a biological explanation. It is known that patients with cancer, including those with ALL, have an increased expression of extracellular vesicles during treatment, which might contribute to the pathogenesis of VTE [44]. It has been shown that children with ALL have an increased level of apoptotic, platelet-derived, and endothelial-derived TF-positive microparticles, as well as increased levels of TF activity and procoagulant phospholipids during IT [45,46]. Furthermore, an increase in platelet-derived microparticles enhancing TG *in vitro* was also reported by Pluchart et al. [44] using Jurkat ALL leukemia cell line. According to published data, TD appears to be more sensitive to microparticles of various origins and circulating procoagulant material [47,48], which increases TG [49,50]. This sensitivity is probably due to the technique, which is based on TF fixed on a surface rather than in solution (as in CAT and STG), which allows for observation of the propagation phase (independent of TF) and, therefore, the contribution that microparticles might have on coagulation during this phase [48]. In addition, TD assay has been shown to distinguish hypo- and more importantly hypercoagulant from normal plasma [51]. Overall, TD was revealed to be a more sensitive assay to capture changes in TG, assessed by ETP, compared with CAT and the automated STG technique.

4.2 | Procoagulant and anticoagulant factors do not capture the early increase in TG

Single procoagulant factors, including VWF and FVIII, were significantly higher in patients with ALL at day 0 compared with healthy controls (Figure 4 and Supplementary Table S1). However, they did not show any difference during treatment despite their function in the coagulation cascade, their known sensitivity to inflammation, and endothelial and platelet activation [3]. Overall, our findings are in line

with previous data showing increased levels of a single procoagulant factor (in particular, VWF and FVIII) at day 0 compared with control group, with considerable variability in the response to therapy [3]. Therefore, changes in TG in patients with ALL cannot be predicted by measuring changes in single plasma procoagulant factors during treatment.

In patients with ALL, we observed a transient increase followed by a statistically significant decrease of the natural anticoagulant factors AT, PC, and PS [3,52]. In agreement with previous data [34], we observed a relevant increase of AT, PC, and PS during the first period (between day 0 and days 8 to 12) of IT, where only prednisone is part of the treatment (Figure 4, Supplementary Table S1). The exact effect of dose, duration, and type of steroid therapy on the hemostatic system is not completely elucidated [3]. In addition, the rise of anticoagulant proteins observed in ALL patients may reflect a compensatory effect of an increased TG due to the disease itself besides the presence of steroids in the initial phase of treatment [7,53]. For instance, it has been suggested that in patients with Cushing's syndrome, who have a chronic excess of cortisol, the rise of AT, PC, and PS could be a secondary event to contrast the increased plasma coagulation activity due to higher levels of clotting factors (FV and FVIII) observed in these patients [54]. Remarkably, despite the increase of natural anticoagulants and the decrease of fibrinogen, patients already developed a procoagulant profile between day 0 and days 8 to 12, whose underlying mechanisms are unclear considering the absence of significant changes of VWF and FVIII. Afterward, between days 8 to 12 and day 22, we observed a significant decrease in AT, PC, PS, and fibrinogen levels ($P < .0001$ for all comparisons; Figure 4C), which is in relation to the treatment of ALL patients with asparaginase (starting at day 12), as previously described [34]. In line with other studies [33,34], we observed a progressive impairment of the physiological PC/PS pathway, as indicated by the trend observed for the median value of TM-dependent ETP inhibition (Supplementary Figure S2), which reflects the effect of PEG-asparaginase on the natural anticoagulant PC and PS, concomitant with the persistently high level of FVIII:C observed in our cohort.

4.3 | Thrombodynamics visualizes an early increase in FCF

In addition to TG, TD can visualize FCF as well. As indicated in Figure 3 and Supplementary Table S2, FCF was already increased in patients at day 0 compared with controls, which is in line with the procoagulant state of children with ALL. Overall, we were able to demonstrate that FCF assessed by TD significantly increases during IT of patients with ALL.

TD provides a real time visualisation of clot growth and quantifies spatio-temporal parameters of *ex vivo* FCF. Employing this technique, we show for the first time that FCF (as measured by Vi, V and CS) is markedly accelerated and increased in children with ALL as the treatment progressed, as indicated by the significant increase in the velocity of clot growth (Vi and V) and the significant increase in CS, both observed already very early between day 0 and days 8 to 12 (Figure 3A–C, Supplementary Table S2). Furthermore, we observed a shortening of the time needed for the appearance of spontaneous clots (Tsp) in patients with ALL compared with controls (Figure 3D). This indicates high thrombin activity and strong hypercoagulability in the bulk of plasma not related to the main clot triggered by the coated TF. Spontaneous clotting, highly present in plasma from patients with ALL during TD analysis, probably depends on circulating microparticles [47], as it was previously observed in plasma of patients with various inflammatory diseases compared with healthy donors [47,55].

The enhanced FCF cannot be attributed to changes in fibrinogen level. Indeed, fibrinogen decreased during IT (Figure 4C and Supplementary Table S1), while FCF and diffuse Tsp both increased (Figure 3). Since DD, an indicator of fibrin degradation, significantly decreased (Figure 1C), we hypothesize that a decreased fibrinolytic activity may play a causal role in the enhanced FCF. This is in line with the paradoxically lower DD measured at day 0 in patients with VTE (median, 840 μg/L; IQR, 466-1044) compared with NoVTE (median, 2269 μg/L; IQR, 1251-6893; $P < .01$). From a practical point of view, DD cannot serve as a sensitive marker to assess the prothrombotic state in patients with ALL. On the contrary, FCF assessed by TD appears to be very sensitive to changes in coagulation state in pediatric patients with ALL.

4.4 | Early increased procoagulant state in patients who later developed VTE

Most interestingly, we observed that 5 patients, later diagnosed with venous thrombotic complication between days 29 and 58, had a significantly higher TG already at days 8 to 12 compared with patients without thrombotic complications (Figure 5, Supplementary Table S3). This observation has mechanistic and prognostic implications. A hypothesis for explaining the TG increase observed on days 8 to 12 is the influence of procoagulant elements, such as cell-free DNA and microparticles, which are liberated during blast lysis induced by corticosteroid therapy [56]. Another possible mechanism may be mediated by platelets and red blood cells [57]. Interestingly, we observed significantly increased hematocrit (mean difference, 10.6 l/L; 95% CI, 5-16) and hemoglobin levels (mean difference, 30 g/L; 95% CI, 13-47) prior to treatment in patients with ALL who later developed thrombosis compared with those who did not. Previous studies in adults and children have shown a correlation between hematocrit level and risk of venous and arterial thrombosis [58,59]; however, not specifically in patients with ALL. Moreover, although the difference was not statistically significant, children with VTE showed increased platelet counts prior to starting treatment compared with children without VTE (mean difference, 79×10^9/L; 95% CI, −40 to 200).

From a clinical point of view, the increased TG measured early in the therapy of patients with ALL by GCAs is promising and may allow early detection of patients at highest risk of VTE. It is important to note that this study was conducted with a small sample size. Further research with larger cohorts is needed to validate and establish the

clinical utility of GCAs in patients with ALL. In addition, although TD provides an additional angle of analysis by allowing spatiotemporal visualization of TG and FCF [25–27], this technique has a limitation since it cannot be used in presence of lipemic plasma. In fact, the device cannot read the contrasts in the light scattering images between the growing clot and the bulk of plasma if the plasma is too dense. Finally, since we defined day 0 within 24 hours from treatment start, 5 out of 15 patients had already received blood transfusion (packed red blood cells and/or platelets concentrates), potentially impacting cell counts. However, none of them received plasma replacement, and GCAs performed in PPP were not affected.

5 | CONCLUSION AND CLINICAL IMPACT

Our data confirm that during IT, children with ALL develop a prothrombotic state characterized by increased TG and show, for the first time, an enhanced FCF as well. Our pilot study shows that GCAs can detect an early procoagulant state in ALL patients and that early changes appear to be able to predict which patients will develop a thromboembolic event. This finding needs to be confirmed in a larger cohort in order to establish GCAs' clinical significance and their prognostic utility, and therefore, we advocate for international collaborations to move forward.

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AUTHOR CONTRIBUTIONS

C.B. performed research, collected laboratory data, analyzed results, and wrote and critically revised the manuscript; D.B.C. contributed to study design, performed and supervised research, collected laboratory data, analyzed results, and wrote and critically revised the manuscript; A.A. contributed to data analysis and critically revised the manuscript; E.L. coordinated sample and patient data collections and critically revised the manuscript; E.M-G. and F.J.G. performed routine laboratory analyses; F.C. contributed to study design, supervised patient treatment, and critically revised the manuscript; L.A. contributed to study design, supervised research and laboratory assays, analyzed the data, and wrote and revised the manuscript. M.R. designed the study, supervised patient treatment, analyzed the clinical and research data, and wrote and revised the manuscript.

DECLARATION OF COMPETING INTERESTS

All authors have no competing interests to disclose.

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REFERENCES

[1] Albisetti M, Kellenberger CJ, Bergstrasser E, Niggli F, Kroiss S, Rizzi M, Schmugge M. Port-a-cath-related thrombosis and post-thrombotic syndrome in pediatric oncology patients. *J Pediatr*. 2013;163:1340–6.

[2] Pelland-Marcotte MC, Pole JD, Kulkarni K, Athale U, Stammers D, Sabapathy C, Halparin J, Brandao LR, Sung L. Thromboembolism incidence and risk factors in children with cancer: a population-based cohort study. *Thromb Haemost*. 2018;118:1646–55.

[3] Giordano P, Molinari AC, Del Vecchio GC, Saracco P, Russo G, Altomare M, Perutelli P, Crescenzio N, Santoro N, Marchetti M, De Mattia D, Falanga A. Prospective study of hemostatic alterations in children with acute lymphoblastic leukemia. *Am J Hematol*. 2010;85:325–30.

[4] Greiner J, Schrappe M, Claviez A, Zimmermann M, Niemeyer C, Kolb R, Eberl W, Berthold F, Bergstrasser E, Gnekow A, Lassay E, Vorwerk P, Lauten M, Sauerbrey A, Rischewski J, Beilken A, Henze G, Korte W, Moricke A, THROMBOTECT Study Investigators. THROMBOTECT—a randomized study comparing low molecular weight heparin, antithrombin and unfractionated heparin for thromboprophylaxis during induction therapy of acute lymphoblastic leukemia in children and adolescents. *Haematologica*. 2019;104:756–65.

[5] Bushman JE, Palmieri D, Whinna HC, Church FC. Insight into the mechanism of asparaginase-induced depletion of antithrombin III in treatment of childhood acute lymphoblastic leukemia. *Leuk Res*. 2000;24:559–65.

[6] Hernandez-Espinosa D, Minano A, Ordonez A, Mota R, Martinez-Martinez I, Vicente V, Corral J. Dexamethasone induces a heat-stress response that ameliorates the conformational consequences on antithrombin of L-asparaginase treatment. *J Thromb Haemost*. 2009;7:1128–33.

[7] Rodriguez V. Thrombosis complications in pediatric acute lymphoblastic leukemia: risk factors, management, and prevention: is there any role for pharmacologic prophylaxis? *Front Pediatr*. 2022;10:828702.

[8] Truelove E, Fielding AK, Hunt BJ. The coagulopathy and thrombotic risk associated with L-asparaginase treatment in adults with acute lymphoblastic leukaemia. *Leukemia*. 2013;27:553–9.

[9] Athale UH, Chan AK. Thrombosis in children with acute lymphoblastic leukemia. Part II. Pathogenesis of thrombosis in children with acute lymphoblastic leukemia: effects of the disease and therapy. *Thromb Res*. 2003;111:199–212.

[10] Payne JH, Vora AJ. Thrombosis and acute lymphoblastic leukaemia. *Br J Haematol*. 2007;138:430–45.

[11] O'Brien SH, Rodriguez V, Lew G, Newburger JW, Schultz CL, Orgel E, Derr K, Ranalli MA, Esbenshade AJ, Hochberg J, Kang HJ, Dinikina Y, Mills D, Donovan M, Dyme JL, Favatella NA, Mitchell LG, PREVAPIX-ALL investigators. Apixaban versus no anticoagulation for the prevention of venous thromboembolism in children with newly diagnosed acute lymphoblastic leukaemia or lymphoma (PREVAPIX-ALL): a phase 3, open-label, randomised, controlled trial. *Lancet Haematol*. 2024;11:e27–37.

[12] De Stefano V, Sora F, Rossi E, Chiusolo P, Laurenti L, Fianchi L, Zini G, Pagano L, Sica S, Leone G. The risk of thrombosis in patients with acute leukemia: occurrence of thrombosis at diagnosis and during treatment. *J Thromb Haemost*. 2005;3:1985–92.

[13] Lejhancova-Tousovska K, Zapletal O, Vytiskova S, Strbackova P, Sterba J. Profile of thrombin generation in children with acute lymphoblastic leukemia treated by Berlin-Frankfurt-Munster (BFM) protocols. *Blood Coagul Fibrinolysis*. 2012;23:144–54.
[14] Burley K, Salem J, Phillips T, Reilly-Stitt C, Marks DI, Tunstall O, Moppett J, Mumford A, Bradbury CA. Evaluation of coagulopathy before and during induction chemotherapy for acute lymphoblastic leukaemia, including assessment of global clotting tests. *Blood Cancer J*. 2017;7:e574.
[15] Lecut C, Peters P, Massion PB, Gothot A. [Is there a place for thrombin generation assay in routine clinical laboratory?]. *Ann Biol Clin (Paris)*. 2015;73:137–49.
[16] Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost*. 2003;1:1504–14.
[17] Tripodi A. Thrombin generation: a global coagulation procedure to investigate hypo- and hyper-coagulability. *Haematologica*. 2020;105:2196–9.
[18] Tripodi A. Thrombin generation assay and its application in the clinical laboratory. *Clin Chem*. 2016;62:699–707.
[19] Gerotziafas GT, Depasse F, Busson J, Leflem L, Elalamy I, Samama MM. Towards a standardization of thrombin generation assessment: the influence of tissue factor, platelets and phospholipids concentration on the normal values of thrombogram-thrombinoscope assay. *Thromb J*. 2005;3:16.
[20] Duarte RCF, Ferreira CN, Rios DRA, Reis HJD, Carvalho MDG. Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations. *Rev Bras Hematol Hemoter*. 2017;39:259–65.
[21] 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.
[22] Lipets EN, Ataullakhanov FI. Global assays of hemostasis in the diagnostics of hypercoagulation and evaluation of thrombosis risk. *Thromb J*. 2015;13:4.
[23] Hemker HC, Wielders S, Kessels H, Beguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thromb Haemost*. 1993;70:617–24.
[24] Giesen PLA, Gulpen AJW, van Oerle R, Ten Cate H, Nagy M, Spronk HMH. Calibrated automated thrombogram II: removing barriers for thrombin generation measurements. *Thromb J*. 2021;19:60.
[25] Bertaggia Calderara D, Zermatten MG, Aliotta A, Batista Mesquita Sauvage AP, Carle V, Heinis C, Alberio L. Tissue factor-independent coagulation correlates with clinical phenotype in factor XI deficiency and replacement therapy. *Thromb Haemost*. 2021;121:150–63.
[26] Sinauridze EI, Vuimo TA, Tarandovskiy ID, Ovsepyan RA, Surov SS, Korotina NG, Serebriyskiy II, Lutsenko MM, Sokolov AL, Ataullakhanov FI. Thrombodynamics, a new global coagulation test: measurement of heparin efficiency. *Talanta*. 2018;180:282–91.
[27] Tuktamyshov R, Zhdanov R. The method of in vivo evaluation of hemostasis: spatial thrombodynamics. *Hematology*. 2015;20:584–6.
[28] ClinicalTrials.gov. International collaborative treatment protocol for children and adolescents with acute lymphoblastic leukemia. http://clinicaltrials.gov/study/NCT01117441; 2022. [accessed December 21, 2023].
[29] ClinicalTrials.gov. Treatment protocol for children and adolescents with acute lymphoblastic leukemia—AIEOP-BFM ALL 2017. https://clinicaltrials.gov/ct2/show/NCT03643276; 2023. [accessed December 21, 2023].
[30] Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoord R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33:4–15.
[31] Spronk HM, Dielis AW, Panova-Noeva M, van Oerle R, Govers-Riemslag JW, Hamulyák K, Falanga A, Cate HT. Monitoring thrombin generation: is addition of corn trypsin inhibitor needed? *Thromb Haemost*. 2009;101:1156–62.
[32] Binder NB, Depasse F, Mueller J, Wissel T, Schwers S, Germer M, Hermes B, Turecek PL. Clinical use of thrombin generation assays. *J Thromb Haemost*. 2021;19:2918–29.
[33] Staddon JH, Smock KJ, Schiffman JD, Fluchel MN, Engel ME, Weyrich AS, Campbell RA. Pegasparaginase treatment alters thrombin generation by modulating the protein C and S system in acute lymphoblastic leukaemia/lymphoma. *Blood Coagul Fibrinolysis*. 2015;26:840–3.
[34] Rozen L, Noubouossie D, Dedeken L, Huybrechts S, Le PQ, Ferster A, Demulder A. Different profile of thrombin generation in children with acute lymphoblastic leukaemia treated with native or pegylated asparaginase: a cohort study. *Pediatr Blood Cancer*. 2017;64:294–301.
[35] Appel IM, Hop WC, Pieters R. Changes in hypercoagulability by asparaginase: a randomized study between two asparaginases. *Blood Coagul Fibrinolysis*. 2006;17:139–46.
[36] Appel IM, van Kessel-Bakvis C, Stigter R, Pieters R. Influence of two different regimens of concomitant treatment with asparaginase and dexamethasone on hemostasis in childhood acute lymphoblastic leukemia. *Leukemia*. 2007;21:2377–80.
[37] Athale U, Moghrabi A, Nayiager T, Delva YL, Thabane L, Chan AK. von Willebrand factor and thrombin activation in children with newly diagnosed acute lymphoblastic leukemia: an impact of peripheral blasts. *Pediatr Blood Cancer*. 2010;54:963–9.
[38] Mitchell L, Hoogendoorn H, Giles AR, Vegh P, Andrew M. Increased endogenous thrombin generation in children with acute lymphoblastic leukemia: risk of thrombotic complications in L'Asparaginase-induced antithrombin III deficiency. *Blood*. 1994;83:386–91.
[39] Ishihara T, Nogami K, Ochi S, Ishida T, Kosaka Y, Sawada A, Inoue M, Osone S, Imamura T, Hosoi H, Shima M. Disordered hemostasis associated with severely depressed fibrinolysis demonstrated using a simultaneous thrombin and plasmin generation assay during L-asparaginase induction therapy in pediatric acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2020;67:e28016.
[40] Depasse F, Binder NB, Mueller J, Wissel T, Schwers S, Germer M, Hermes B, Turecek PL. Thrombin generation assays are versatile tools in blood coagulation analysis: a review of technical features, and applications from research to laboratory routine. *J Thromb Haemost*. 2021;19:2907–17.
[41] Zermatten MG, Fraga M, Calderara DB, Aliotta A, Moradpour D, Alberio L. Biomarkers of liver dysfunction correlate with a prothrombotic and not with a prohaemorrhagic profile in patients with cirrhosis. *JHEP Rep*. 2020;2:100120.
[42] Kumar R, Katare PB, Lentz SR, Modi AJ, Sharathkumar AA, Dayal S. Thrombotic potential during pediatric acute lymphoblastic leukemia induction: role of cell-free DNA. *Res Pract Thromb Haemost*. 2021;5:e12557.
[43] Kuprash AD, Shibeko AM, Vijay R, Nair SC, Srivastava A, Ataullakhanov FI, Panteleev MA, Balandina AN. Sensitivity and robustness of spatially dependent thrombin generation and fibrin clot propagation. *Biophys J*. 2018;115:2461–73.
[44] Pluchart C, Barbe C, Poitevin G, Audonnet S, Nguyen P. A pilot study of procoagulant platelet extracellular vesicles and P-selectin increase during induction treatment in acute lymphoblastic leukaemia paediatric patients: two new biomarkers of thrombogenic risk? *J Thromb Thrombolysis*. 2021;51:711–9.
[45] Schneider P, Van Dreden P, Rousseau A, Kassim Y, Legrand E, Vannier JP, Vasse M. Increased levels of tissue factor activity and procoagulant phospholipids during treatment of children with acute lymphoblastic leukaemia. *Br J Haematol*. 2010;148:582–92.
[46] Yenigurbuz FD, Kizmazoglu D, Ates H, Erdem M, Tufekci O, Yilmaz S, Oren H. Analysis of apoptotic, platelet-derived, endothelial-derived, and tissue factor-positive microparticles of children with acute

lymphoblastic leukemia during induction therapy. *Blood Coagul Fibrinolysis*. 2019;30:149–55.
[47] Lipets E, Vlasova O, Urnova E, Margolin O, Soloveva A, Ostapushchenko O, Andersen J, Ataullakhanov F, Panteleev M. Circulating contact-pathway-activating microparticles together with factors IXa and XIa induce spontaneous clotting in plasma of hematology and cardiologic patients. *PLoS One*. 2014;9:e87692.
[48] Lipets EN, Antonova OA, Shustova ON, Losenkova KV, Mazurov AV, Ataullakhanov FI. Use of Thrombodynamics for revealing the participation of platelet, erythrocyte, endothelial, and monocyte microparticles in coagulation activation and propagation. *PLoS One*. 2020;15:e0227932.
[49] Gasa N, Meiring M. Microparticles: a link to increased thrombin generation. *Blood Coagul Fibrinolysis*. 2021;32:204–8.
[50] Van Der Meijden PE, Van Schilfgaarde M, Van Oerle R, Renne T, ten Cate H, Spronk HM. Platelet- and erythrocyte-derived microparticles trigger thrombin generation via factor XIIa. *J Thromb Haemost*. 2012;10:1355–62.
[51] Bertaggia Calderara D, Aliotta A, Zermatten MG, Kroll D, Stirnimann G, Alberio L. Hyper-coagulability in obese patients accurately identified by combinations of global coagulation assay parameters. *Thromb Res*. 2020;187:91–102.
[52] Zakarija A, Kwaan HC. Adverse effects on hemostatic function of drugs used in hematologic malignancies. *Semin Thromb Hemost*. 2007;33:355–64.
[53] Mall V, Thomas KB, Sauter S, Niemeyer CM, Sutor AH. Effect of glucocorticoids, E. coli- and Erwinia L-asparaginase on hemostatic proteins in children with acute lymphoblastic leukemia. *Klin Padiatr*. 1999;211:205–10.
[54] Kastelan D, Dusek T, Kraljevic I, Polasek O, Giljevic Z, Solak M, Salek SZ, Jelcic J, Aganovic I, Korsic M. Hypercoagulability in Cushing's syndrome: the role of specific haemostatic and fibrinolytic markers. *Endocrine*. 2009;36:70–4.
[55] Peshkova AD, Evdokimova TA, Sibgatullin TB, Ataullakhanov FI, Litvinov RI, Weisel JW. Accelerated spatial fibrin growth and impaired contraction of blood clots in patients with rheumatoid arthritis. *Int J Mol Sci*. 2020;21:9434.
[56] Del Principe MI, Del Principe D, Venditti A. Thrombosis in adult patients with acute leukemia. *Curr Opin Oncol*. 2017;29:448–54.
[57] Sun S, Campello E, Zou J, Konings J, Huskens D, Wan J, Fernandez DI, Reutelingsperger CPM, Ten Cate H, Toffanin S, Bulato C, de Groot PG, de Laat B, Simioni P, Heemskerk JWM, Roest M. Crucial roles of red blood cells and platelets in whole blood thrombin generation. *Blood Adv*. 2023;7:6717–31.
[58] Brotschi B, Hug MI, Latal B, Neuhaus D, Buerki C, Kroiss S, Spoerri C, Albisetti M. Incidence and predictors of indwelling arterial catheter-related thrombosis in children. *J Thromb Haemost*. 2011;9:1157–62.
[59] Folsom AR, Wang W, Parikh R, Lutsey PL, Beckman JD, Cushman M, Atherosclerosis Risk in Communities (ARIC) Study Investigators. Hematocrit and incidence of venous thromboembolism. *Res Pract Thromb Haemost*. 2020;4:422–8.

SUPPLEMENTARY MATERIAL

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