Screening for Coagulopathy and Identification of Children With Acute Lymphoblastic Leukemia at a Higher Risk of Symptomatic Venous Thrombosis: An AIEOP Experience

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Introduction: Venous thromboembolic events (VTEs) are frequent complications of childhood acute lymphoblastic leukemia (ALL) treatment. The aim of the study was to evaluate the rate of symptomatic VTEs in children with ALL and the predictive value of clinical and biological factors and routine monitoring of coagulation parameters in identifying children at a higher risk of this complication.

Materials and Methods: Between September 2000 and July 2006, 2042 children (≥ 1 and younger than 18 y) with newly diagnosed ALL were enrolled in Italy in the AIEOP (Italian Association of Pediatric Hematology and Oncology)-BFM (Berlin-Frankfurt-Muenster) ALL 2000 trial. Patients with symptomatic VTEs (deep venous thromboses or cerebral venous thromboses) were identified after a careful review of clinical records. The impact of coagulation derangement at the onset of VTEs was evaluated by a nested case-control study.

Results: Forty-eight (2.4%) children presented with a VTE. The rate of VTEs was higher in male patients ($P = 0.001$); patients randomized to receive dexamethasone tended to have a higher rate of VTE compared with those who received prednisone ($P = 0.10$). The coagulation derangement at the onset of VTE was not associated with VTE occurrence. The prevalence of a factor V Leiden G1691A mutation and the prothrombin G20210A variant was higher in children with VTE than that expected in the general population.

Key Words: acute lymphoblastic leukemia, childhood, L-asparaginase, screening of coagulopathy, treatment of thrombosis

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Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy.[1] The use of aggressive polychemotherapy protocols in the last 2 to 3 decades has determined a steady improvement of outcome in children with ALL, ALL now being a curable disease for the large majority of pediatric patients.[2]

Venous thromboembolic events (VTEs) may occur during childhood ALL treatment as a result of the interaction between different factors such as the disease itself, the use of a central venous line (CVL), inherited predisposing factors, and chemotherapy.[3–6] VTE can impact the treatment and the outcome adversely; its severity may be very different, ranging from asymptomatic cases to death.[3]

The reported rate of VTEs in children with ALL varies from 0.95% to 36.7%[3,7,8]; this huge variation is related to the definition of VTE (symptomatic vs. asymptomatic), diagnostic methods, treatment protocols, flaws in data reporting and nature (either prospective or retrospective) of the study.[3]

A previous retrospective study conducted in Italy showed a 0.95% prevalence of symptomatic VTEs among 2318 children with ALL treated according to the Italian Association of Pediatric Hematology and Oncology (AIEOP) ALL 91 and 95 protocols.[8] In that study, the T-immunophenotype was associated with an increased risk of VTE.

The AIEOP-Berlin-Frankfurt-Muenster (BFM) ALL 2000 study protocol for childhood ALL is based on the back-bone of the BFM ALL protocols.[2,9] To better evaluate the rate and the outcome of adverse events in AIEOP patients, a web-based remote data entry system for the data collection of enrolled patients was introduced in Italy since 2000.

Any event reported in this system was reviewed routinely by a panel of experts in order to define controversial aspects of diagnosis, pathogenesis, and outcome.

The main objectives of this study were to evaluate (i) the rate of VTE in the population of Italian children with ALL enrolled in the AIEOP-BFM ALL 2000 protocol, (ii) the relationship between VTE onset and the type of steroid [prednisone (PDN) vs. dexamethasone (DXM)] used in a randomized manner during induction phase, and (iii) the predictive value of several clinical and biological factors (including the coagulation status through a case-control study) on the risk of VTE occurrence.

MATERIALS AND METHODS

Between September 2000 and July 2006, a total of 2042 children aged 1 year and above and below 18 years with ALL, consecutively diagnosed and treated in 35 AIEOP centers, were eligible and evaluable for the AIEOP-BFM ALL 2000 protocol.[2,9,10] The entire cohort is analyzed here.

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The initial notification of suspected VTE was included in a specific database created to collect and evaluate the adverse events occurring during protocol application. This web-based remote data entry system allowed the prospective collection of not only the usual demographic information, biological characteristics of the disease, the pattern of response to treatment, doses of chemotherapeutic drugs, and follow-up data but also toxicity data and adverse events occurring during chemotherapy treatment delivery.

A Survey on VTE

After suspected VTE notification, a review process was started, with a specific survey conducted by sending a questionnaire to the treating physicians to obtain additional information that was not a part of the routine data collection, including the type of L-asparaginase (L-ASP) used during the induction phase, the coagulation tests performed at the time of VTE onset, the coagulation status at VTE onset in terms of platelet counts, prothrombin activity, and levels of fibrinogen and antithrombin III. Factors potentially contributing to the increased risk of VTE were classified as acquired (L-ASP source, steroid type, presence of a CVL, sepsis, shock, dehydration, and liver or renal insufficiency) or inherited [the presence of factor V Leiden, G20210A mutation in the prothrombin gene and T677T homozygous status of the methylenetetrahydrofolate reductase (MTHFR) mutated gene]. In general, these genetic data were obtained after a VTE was diagnosed; the decision to screen for inherited prothrombotic factors was left to the treating physician. Data about instrumental investigations used for VTE diagnosis were also recorded.

The clinical outcome was observed for at least 3 months after VTE onset and was classified as favorable (no deficits or symptoms) or unfavorable (persistence of 1 or more deficits or symptoms or death). Causes of death were also reviewed.

In the present study, only symptomatic VTEs, further classified as deep venous thromboses (DVT) or cerebral venous thromboses (CVT) according the primary site of VTE onset, were included. A symptomatic VTE was defined as any VTE diagnosed by appropriate instrumental testing after the appearance of clinical symptoms typically suggesting VTE. CVL malfunctioning because of clots was not considered as a VTE.

A Case-Control Study on VTE

In order to evaluate the association between coagulation factors and VTE, a nested case-control study was designed specifically in the AIEOP-ALL 2000 cohort. A set of 2 controls was extracted from the cohort and matched to each VTE case according to the following criteria: sex, age (1 to 5, 6 to 9, ≥ 10 y), the immunophenotype (T-ALL, pB-ALL), the steroid used (DXM, PDN, not randomized), the phase of therapy, and alive (and in complete remission when applicable) at the same time of scheduled chemotherapy with respect to the case patient.

The AIEOP-BFM ALL 2000 multicenter study was conducted in accordance with the ethical standards stated in the Declaration of Helsinki and approved by the local ethical committee of each participating institution; informed consent was obtained from parents or guardians.

Treatment

In the AIEOP-BFM ALL 2000 protocol,[2,9,10] all patients received a 7-day-long prephase with oral PDN (60 mg/m^2) and 1 dose of intrathecal (IT) methotrexate (MTX) on day 1. The induction phase (ie, protocol I) was divided into 2 phases called Ia and Ib. In phase Ia, patients were randomized to receive either PDN (60 mg/m^2) or dexametazone (DXM, 10 mg/m^2) in 3 divided doses from day 8 to 29 (then tapered over 9 d). In addition, all patients received the following: L-ASP (in this protocol, the native L-ASP *Escherichia coli* product was used, given IM at a dosage of 5000 IU/m^2, at 3-day intervals, starting on day 12 through day 33; 8 doses); weekly vincristine (VCR; 1.5 mg/m^2 IV) and daunorubicin (30 mg/m^2 IV) on days 8, 15, 22, and 29; and IT MTX on days 15 and 29. In phase Ib, children received daily oral 6-mercaptopurine (60 mg/m^2) from day 36 to 63, cyclophosphamide (CPM; 1 g/m^2 IV) on days 36 and 64, daily cytosine arabinoside IV or SC (ARA-C, 75 mg/m^2) on days 38 to 41, 45 to 48, 52 to 55, and 59 to 62, and IT MTX on days 38 and 52. Bone marrow evaluation was performed on days 33 and 78 for the evaluation of morphologic complete remission and minimal residual disease. Patients were stratified as belonging to the standard risk (SR), the intermediate risk (IR), or the high risk (HR) groups according to the (1) treatment response (PDN response on day 8 of chemotherapy schedule and marrow CR response on days 33 and 78 of the chemotherapy schedule), (2) minimal residual disease evaluation after protocols Ia and Ib, and (3) cytogenetic aberrations [namely, the presence or the absence of t(9;22) and t(4;11)].

SR and IR patients received, after protocol I, a consolidation phase consisting of high-dose methotrexate (HD-MTX, 2 or 5 g/m^2 given over 24 h q 14 d $\times$ 4 cycles), whereas HR patients received 3 high-dose chemotherapy blocks including HD-MTX or HD-ARA-C.

After consolidation, SR patients were randomized to receive a reinduction phase consisting of protocols II or III (details of the treatment have been already described elsewhere[9]). IR patients were randomized to receive a reinduction phase consisting of protocol II once or to receive protocol III twice (a 3-month interval phase consisting of oral 6-mercaptopurine and MTX was given between the 2 protocols III). HR patients were randomized to receive protocol II twice (with a 6-week interval phase, see above for details) or protocol III 3 times (with a 6-week interval phase, see above for details). Protocol II includes the following: oral DXM (10 mg/m^2) on days 1 to 21, and the dose was then tapered; weekly VCR (1.5 mg/m^2 IV) and doxorubicin (ADR; 30 mg/m^2 IV) on days 8, 15, 22, and 29; L-ASP IM at the dosage of 10,000 UI/m^2 on days 8, 11, 15, and 18; CPM (1 g/m^2 IV) on day 36; oral 6-thioguanine (60 mg/m^2) on days 36 to 49; ARA-C (75 mg/m^2 SC or IV) on days 38 to 41 and days 45 to 48; IT MTX on days 38 and 45.

Protocol III includes the following: oral DXM (10 mg/m^2) on days 1 to 14, and the dose was then tapered; weekly VCR (1.5 mg/m^2 IV) and ADR (30 mg/m^2 IV) on days 1 and 8; L-ASP (10,000 UI/m^2 IM) on days 1, 4, 8, and 11; CPM (500 mg/m^2 IV) on day 15; oral 6-thioguanine (60 mg/m^2) on days 15 to 28; ARA-C (75 mg/m^2 IV or SC) on days 16 to 19 and 23 to 26; IT MTX on days 16 and 23.

Indications for replacement therapy (eg, antithrombin, fresh-frozen plasma) and suspension or delay of L-ASP administration based on coagulation or biochemical tests were not provided in the AIEOP-BFM ALL 2000 protocol.

Statistical Analysis

The rate of VTE in different subgroups was compared with parametric tests on proportions. The Cox regression model was applied to investigate the influence of characteristics (sex; age with categories 1 to 5, 6 to 9, and ≥ 10 y; and immunophenotype, Bcp vs. T-ALL) and treatment (DXM vs. PDN in induction) on the risk of developing a VTE. Confidence intervals for prevalence of genetic prothrombotic factors were calculated according to Wilson.[11]

The matched case-control study (48 cases and 96 controls) had an 80% power to show a decrease in the proportion of patients with coagulation problems (fibrinogen < 100 mg/dL; antithrombin < 80 U/mL) from 40% to 17% ($\alpha = 0.05$). The coagulation status was assessed in all cases according to the 2 parameters considered, and a platelet count was performed. When these data were missing for both matched controls, the patients (cases and controls) were excluded from the analysis.

In the case-control study, a χ^2 test was used to assess whether VTE cases and controls differed in their levels of fibrinogen and AT, whereas the Wilcoxon test was used to compare platelet counts in the 2 groups. A significance level of 0.05 was considered (2 sided).

RESULTS

Overall, a total of 63 cases initially reported with suspected VTE were reviewed in order to confirm the diagnosis. Of these, 11 cases were classified after the review process as follows: brain artery thrombosis (n = 2); leucoencephalopathy (n = 2); clots and malfunctioning of CVL, without any evidence or symptoms of thrombosis (n = 7). For 4 additional cases, data available to confirm the diagnosis of VTE were insufficient.

Thus, the 48 documented VTE episodes that were observed among the 2042 patients (2.4%) enrolled by the AIEOP centers in the AIEOP ALL-BFM 2000 study are reported here. Clinical and laboratory features (sex, age, immunophenotype, and the type of steroid used after the prephase) are reported in Table 1. Additional relevant features (the phase of treatment, the site of VTE, the type of L-ASP used, CVL in situ, and genetic prothrombotic risk) collected in this group of patients are reported in Table 2. None of the patients received thrombosis prophylaxis with low-molecular-weight heparin during chemotherapy.

Of the 48 VTEs, 33 were CVTs and 15 were DVTs (10 of the upper venous system, and 5 of the lower venous system); no patients had pulmonary embolism.

The rate of VTE was 3.3% in male patients (38/1144) and 1.1% in female patients (10/898; $P = 0.001$). According to the immunophenotype, the rate of VTE in T versus non-T ALL patients was 2.4% (6/246) versus 2.3% (41/1768; $P = 0.91$), (the immunophenotype was not known for 1 patient) and similarly, no significant difference in the VTE rate was found in subgroups identified according to age and WBC at diagnosis.

Sex remained significantly related to the risk of developing VTE even after adjusting for all the other characteristics and for the treatment administered in a Cox regression model. A subanalysis performed on 1856 patients after excluding those who did not use *E. coli* ASP during induction or who suffered from severe liver disease or septicemia yielded superimposable results.

TABLE 1. Clinical and Laboratory Features of 48 and 1994 ALL Children Enrolled in the AIEOP-BFM ALL 2000 Study With or Without a VTE, respectively

	N (%)		
	VTE	**No VTE**	***P***
Total	48 (2.4)	1994 (97.6)	
Sex			
Male	38 (3.3)	1106 (96.7)	0.001
Female	10 (1.1)	888 (98.9)	
Age (y)			
1-5	23 (1.9)	1186 (98.1)	0.22
6-9	11 (2.7)	401 (97.3)	
10-17	14 (3.3)	407 (96.7)	
WBC			
< 20000	33 (2.6)	1261 (97.4)	0.50
20-100,000	9 (1.7)	522 (98.3)	
≥ 100,000	6 (2.8)	210 (97.2)	
Not known	– (–)	1 (–)	
Immunophenotype			
B-cell precursor ALL	41 (2.3)	1727 (97.7)	0.91
T-ALL	6 (2.4)	240 (97.6)	
Not known	1 (–)	27 (–)	
Randomized patients			
DXM	20 (2.8)	684 (97.2)	0.10
PDN	12 (1.6)	750 (98.4)	
Not randomized	16 (2.8)	560 (97.4)	

AIEOP-BFM indicates Association of Pediatric Hematology and Oncology-Berlin-Frankfurt-Muenster; ALL, acute lymphoblastic leukemia; DXM, dexametazone; PDN, prednisone; VTE, venous thromboembolic event.

The Type of Steroid Administered in Phase Ia

Among the 2042 patients enrolled in the protocol, 1466 patients were randomized, immediately after the PDN steroid prephase, to receive either PDN (n = 762) or DXM (n = 704) during phase Ia; 576 patients were not randomized (565 patients received PDN, 8 received DXM, and 3 patients died before the randomization). The rate of VTE among patients receiving PDN or DXM was 1.6% (12/762) versus 2.8% (20/704; $P = 0.10$), whereas the rate in non-randomized patients was 2.8% (16/576).

The Treatment Phase

It is of note that almost 2/3 of all VTEs occurred during induction phase Ia (n = 31, 64.6%), 2 (4.2%) occurred during the treatment prephase (7-day-long PDN administration period), and thus before any L-ASP dose was administered (ie, day + 12), 7 (14.6%) during phase Ib, and 8 (16.6%) during the reinduction phase. Figure 1 shows the outline of phase Ia and Ib (induction) and highlights the days when each VTE was observed.

The Type of L-ASP Preparation

Forty-one of the 48 patients with VTE (85.4%) received *E. coli* L-ASP, whereas 3 patients received Erwinia C. L-ASP; this distribution reflected the fact that the vast majority of patients enrolled in Italy in the AIEOP-BFM ALL 2000 study received the *E. coli* ASP product (n = 1929), whereas only a minority (n = 113) received the Erwinia C. ASP product. As a consequence, the type of L-ASP preparation did not impact the risk of VTE onset (*E. coli* ASP 41/1929, that is, 2.1%; Erwinia C. ASP 3/113, i.e., 2.6%). Two additional patients received PEG L-ASP and belonged to a unique cohort of 20 patients who were

TABLE 2. Treatment Details and Additional Clinical and Genetic Features of the 48 ALL Children Presenting With a VTE

	N (%)		
	Overall	**Cerebral**	**DVT**
VTE	48	33 (68.7)	15 (31.2)
Site of thrombosis		Sinuses = 26	Upper venous systems = 10
		Cortical veins = 3	Lower venous systems = 5
	—	Sinuses and cortical veins = 4	
		Multiple sites = 20	
Treatment phase			
Induction (IA)	33*	23*	10
Induction (IB)	7	3	4
Reinduction (II/III)	8	7	1
L-Asparaginase product			
Escherichia coli	41	27	14
Erwinia C.	3	2	1
Pegylated *Escherichia coli*	2	2	0
Not applicable	2*	2*	—
CVL in situ	24	11	13
Genetic prothrombotic factors			
MTHFR T677T genotype (performed in 29 patients)	3† (10.3%‡)	2	1
95% CI	3.6-26.4		
FVL G1691A mutation (performed in 30 patients)	6† (20%§)	3	3
95% CI	9.5-37.3		
Prothrombin G20210A variant (performed in 28 patients)	3 (10.7%‖)	2	1
95% CI	3.7-27.2		

*Two of these venous thromboembolic events occurred in the prephase.

†There were patients with 2 or 3 factors concomitantly present: prothrombin G20210 variant and MTHFR T677T genotype (n = 1); prothrombin G20210 variant, MTHFR T677T genotype and factor V Leiden (n = 1).

‡Expected in general population: 14% to 15%.

§Expected in general population: 3% to 7%.

‖Expected in general population: 1% to 3%.

ALL indicates acute lymphoblastic leukemia; CI, confidence intervals; CVL, central venous line; DVT, deep venous thrombosis; FVL, factor V Leiden; MTHFR, methylen-tetrahydrofolate-reductase; VTE, venous thromboembolic event.

not randomized during induction (they received PDN as the steroid treatment) and were included in a pharmacological study on the drug (the PEG-L-ASP product was administered at 1000 IU/m^2 IV on days 12 and 27 of phase Ia and on day 8 of protocol II). Two patients did not receive any L-ASP product because VTE occurred before L-ASP administration (Table 2). All patients with VTE discontinued the L-ASP treatment planned in the current treatment phase or in the following phases.

The Role of CVL

Twenty-four out of the 48 patients (50%) with VTE had a CVL in situ when the VTE occurred. To address the issue of whether a higher rate was associated with the presence of CVL, we analyzed patients in phase Ia. Out of 2023 patients for whom data were available, 1408 had a CVL during phase Ia, and 9 experienced DVT compared with 1 out of 615 patients without CVL ($P = 0.3$). Seven patients experienced DVT of the upper limbs during induction phase Ia; all had a CVL in situ when the VTE occurred.

Inherited Prothrombotic Risk Factors

Genetically determined prothrombotic risk factors (factor V Leiden, MTHFR 677TT homozygous genotype, prothrombin G20210A variant) were searched after VTE occurrence in 36/48 patients. As shown in Table 2, on the basis of 95% confidence intervals, we showed a significantly higher prevalence of FVL and FII in the ALL population with regard to the general population (where the prevalence is expected to be 3% to 7% and 1% to 3%, respectively). The prevalence of MTHFR homozygosity was not found to be significantly different from that of the general population, as the 95% confidence interval includes the population figure of 14% to 15%.[12]

At VTE onset, 2 patients presented with sepsis and renal insufficiency (grade III, WHO). One additional patient showed concomitant liver insufficiency (grade IV, WHO), renal insufficiency (grade III, WHO), and hyponatremic dehydration (grade III, WHO).

Clinical Features and Symptoms at VTE Onset

DVT

All patients with a DVT (n = 15) occurring either in the upper or in the lower vein system showed typical signs or symptoms such as pain [13/15 (86.6%)] and swelling [11/15 (73.3%)] in the areas involved. Echo-color-doppler was the examination of first choice in all patients and it allowed confirmation of the diagnosis of DVT.

CVT

Among the 33 patients with CVT, the most common neurological symptoms (each patient presented with 1 or more symptoms) were headache (n = 14), a decreased level or loss of consciousness (n = 15), visual impairment (n = 3), and focal or generalized seizures (n = 18); 1 patient showed photophobia; 8 patients presented with vomiting and 3 with irritability. Other signs were hemiparesis (n = 5), ataxia (n = 2), speech impairment (n = 6), and cranial nerve palsy (n = 1).

A brain computed tomography (CT) scan was the examination of first choice in 22/33 (66.6%) cases and magnetic resonance imaging (MRI) in 11 (33.3%) cases; in 16/33 (48.4%) cases, the CT scan was followed by an MRI for diagnostic confirmation. In 2 cases of CVT, CT scan did not allow the diagnosis that was made by magnetic resonance venography.

Overall, MRI was performed in 27/33 (81.8%) patients; in 26/27 (96.2%) cases, magnetic resonance venography was performed, and the diagnosis of CVT was confirmed in all cases.

Coagulation Tests at VTE Onset: Case-Control Analyses

Fibrinogen levels were available in 43 patients and 86 matched controls, and were < 50 mg/dL in 3 VTE patients and in none of the controls. Sixty percent of cases presented with a level of fibrinogen ≥ 100 mg/dL, whereas this was

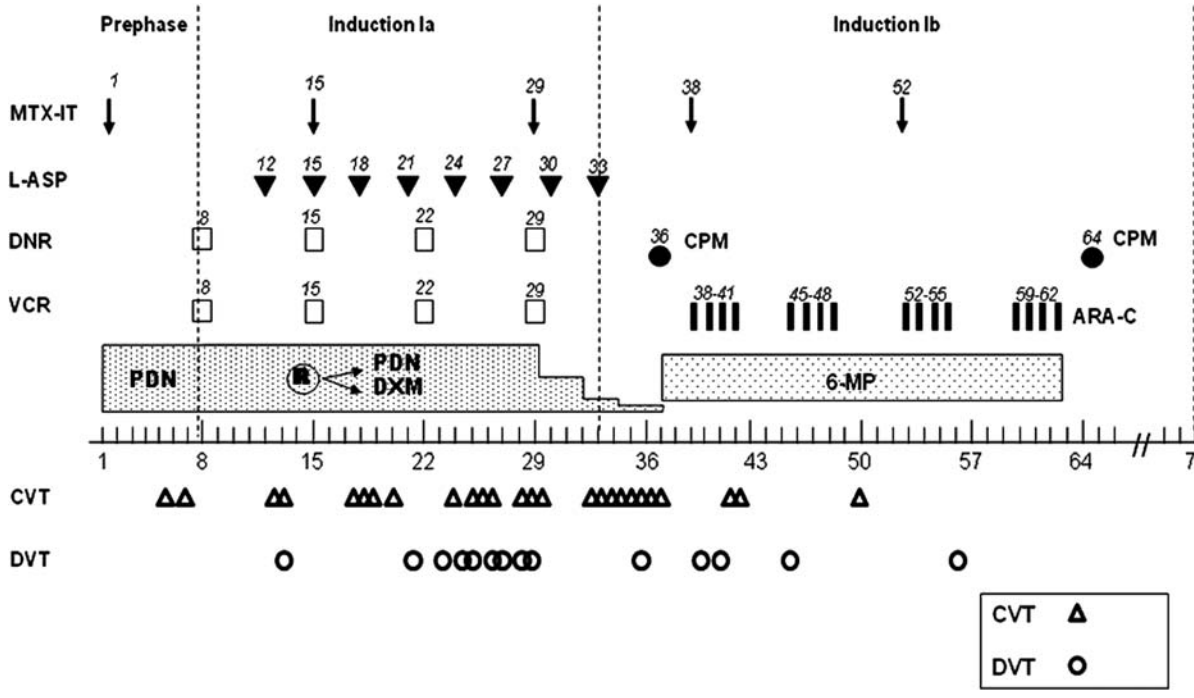

FIGURE 1. Outline of the induction phase with the days where each venous thromboembolic event was observed. 6-MP indicates 6-mercaptopurine; ARA-C, cytosine arabinoside; CPM, cyclophosphamide; CVT, cerebral venous thromboses; DNR, daunorubicin; DVT, deep venous thromboses; DXM, dexamethasone; L-ASP, L-asparaginase; MTX-IT, methotrexate intrathecal; PDN, prednisone; VCR, vincristine.

seen in 69% of the controls, with a nonsignificant difference. Antithrombin III levels were available in 30 patients and 54 matched controls and were < 50 U/mL in 2 (4.7%) cases and in 2 (3.7%) controls; 57% of cases presented with normal levels (≥ 80 U/mL), whereas this was seen in 70% of the controls, the difference being nonsignificant. Also, the mean and the median platelet counts did not differ significantly in 48 cases and 96 controls. Similar results were obtained when the analysis was performed by the type of steroid administered during induction (either DXM or PDN; further details in Table 3).

The Clinical Outcome

No deficits or symptoms persisting at least 3 months after the VTE were observed in 43/48 (89.5%) cases; in 5/48 (10.4%) cases, all with CVT, the outcome was characterized by language impairment (n = 1), drug-resistant epilepsy (n = 1), and death in 3 additional patients (6.2%): 2 of them died of cerebral parenchymal hemorrhage and the third of an untreatable status epilepticus and malignant hypertension.

Incidence of leukemia relapses among the 48 patients with VTE was similar to that observed among the remaining 1994 patients (data not shown).

DISCUSSION

VTE represents a frequent and serious complication in children with ALL. The rate of VTE in children with ALL has been reported to be up to 36.7%, with an overall calculated average of 3.2%.[3] This wide variation in scientific reports may be due to different factors including the relevance of the clinical impact of VTE (symptomatic vs. asymptomatic), diagnostic methods used, the number of patients analyzed, the study design (prospective vs. retrospective analysis), differences in ALL treatment protocols, and flaws in data reporting.[3] In particular, prospectively designed studies reported a higher rate of VTE, even when

TABLE 3. Details of the Case/Control Study Performed on the Coagulation Status

	Case	Control
Fibrinogen (mg/dL)		
No. patients	43	86
Mean (SD)	171 (162)	161 (118)
Median (range)	115 (28-675)	120 (52-753)
Levels		
< 50	3	0
50-100	14	27
≥ 100	26 (60%)	59 (69%)
P-value (< 100 vs. ≥ 100)	0.36	
Platelets (count/mm^3)		
No. patients	48	96
Mean (SD)	177 (113)	197 (121)
Median (range)	144 (7-765)	188 (31-641)
P-value (Wilcoxon)	0.27	
Antithrombin (U/mL)		
No. patients	30	54
Mean (SD)	88 (29)	98 (30)
Median (range)	80 (49-139.3)	94 (44-196.6)
Levels		
< 50	2	2
50-80	11	14
≥ 80	17 (57%)	38 (70%)
P-value (< 80 vs. ≥ 80)	0.21	

similar chemotherapy protocols have been used. In a retrospective survey of a large group of ALL children (n = 1100) treated with the BFM ALL 90 protocol, the rate of VTE reported by Sutor et al[13] was 1.7%, whereas Korte and colleagues in a prospective study on only 21 patients treated with the same protocol reported a 14.3% rate.[3,14] Also, in the prospective Prophylactic Antithrombin Replacement in Kids with ALL treated with Asparaginase study, a very high rate of VTE (36.7%) was reported among 60 children treated for ALL; however, most of those VTEs were asymptomatic and were diagnosed by routine screening with bilateral venography or MRI.[15] Even small variations in the treatment schedule may have an impact on the rate of VTE.[16,17]

The rate of VTE (2.4%) found in our study is quite similar to the average rate (5.2%) of a meta-analytic report including 17 different studies,[18] but is higher than that reported in a previous retrospective AIEOP study performed on 2318 children (0.95%) treated with the previous AIEOP ALL 91 and 95 protocols whose schedules were very similar to the AIEOP ALL-BFM 2000 protocol; a possible explanation for this discrepancy could be the methodology of data collection (prospective in this study vs. retrospective in the previous one).

In the previous AIEOP study on VTE, the T immunophenotype was reported to represent an additional risk factor for thrombosis.[8] Data of the present study do not confirm a significant difference in the rate of VTE among patients with the T-ALL and those with the non-T ALL immunophenotype.

It is unclear whether age is a relevant risk factor for VTE. In the pediatric age group, infants and adolescents are considered to be at a higher risk of developing VTE mainly because of a reduced fibrinolytic potential in infants and because of a physiological reduction of fibrinolytic factors and a reduced response to venous occlusion stress in older children and adolescents.[19–24] Appel and colleagues have demonstrated that children with ALL aged 11 to 16 years show, during chemotherapy treatment, a more severe decline of anticoagulant and fibrinolytic parameters, thus leading to a higher risk of VTE.[25] In our study, higher rates of VTE were associated, although not significantly, with an older age (1 to 5 y: 1.9%; 6 to 9 y: 2.7%; 10 to 17 y: 3.3%; $P = 0.22$, Table 1), confirming the findings of previously mentioned studies.

We observed a significantly higher rate of VTE in male than in female patients [38/1144 (3.3%) vs. 10/898 (1.1%), $P = 0.001$]. A slight, even if nonsignificant, male predominance in children with ALL is also reported in several other studies.[3,17,18,26,27] Pui et al,[28] in particular, have reported a marked preponderance of male patients among ALL children with VTE (8 male patients out of 11 patients with VTE). In contrast, Priest et al[29] have reported a slight female predominance. Also, in the previous AIEOP study, a male predominance among children with VTE was reported.[30]

VTEs in children with ALL occur almost exclusively during early phases of chemotherapy treatment, with the majority of these episodes occurring during the first 5 to 6 weeks of induction.[18,31] This evidence is fully in line with the findings of the present study as 40/48 VTEs occurred during the induction phase. The explanation of this phenomenon is not fully understood, but it is most probably multifactorial; during the induction phase, which usually includes a prolonged treatment with steroids and L-ASP, the prothrombotic status induced by the disease itself adds to the effects of chemotherapy. Recent studies in fact hypothesize that the combination of L-ASP with corticosteroids, rather than the use of L-ASP alone, is more likely one of the main causes of this complication.[3] It is of note that 26 VTEs occurred on days 15 to 36 of the induction phase and 11 VTEs occurred on days of maximum L-ASP activity (days 27 to 33 of the induction phase)[21,31]; therefore, physicians should pay close attention to clinical symptoms during those days of therapy, to achieve early diagnosis of VTEs and to initiate the correct treatment promptly.

Regarding the type of steroid used during the induction phase, a recent meta-analysis of several studies performed prospectively to better understand the phenomenon of VTE in childhood ALL has shown that patients receiving PDN during induction had a higher risk of developing VTE than patients receiving DXM; however, probably because of the small number of patients receiving DXM, this difference was not statistically significant.[18] Our study showed that patients randomized to receive DXM tended to have a higher rate of VTE [n = 20/704 (2.8%)] compared with those who received PDN [n = 12/762 (1.6%)], but this difference was not statistically significant ($P = 0.10$). In a recent study reported by Nowak Göttl and colleagues in 56 children treated in Germany with the AIEOP-BFM ALL 2000 protocol and receiving DXM during induction (10 mg/m^2/d) in combination with *E. coli* L-ASP (5000 IU/m^2 at 3 d interval starting day 12 through day 33, total 8 doses) had a significantly reduced risk of VTE compared with 280 children who received PDN (60 mg/m^2/d during induction) in the previous BFM-ALL 90/95 protocols (which were very similar to the AIEOP-BFM ALL 2000 protocol; DXM 1.8% vs. PDN 10.4% $P = 0.028$).[31] It should be emphasized, however, that the study mentioned above was conducted in subsequent protocols and that the results were based on a historical comparison and not on a prospective randomized trial aimed at comparing the clinical and biological effects of these 2 different steroids.[4,31]

In our study, a number of additional factors potentially contributing to better define the risk of VTE occurrence were studied; among these, we found that factors FVL and FII were significantly more prevalent in our cohort than in the general population, reported to be 3% to 7% and 1% to 3%, respectively. Additional factors investigated were the presence of a CVL and the type of L-ASP; both these factors did not predict an increased risk of VTE. In contrast, considering the fact that 100% of patients with DVT of upper limbs during induction phase Ia had a CVL in situ when VTE occurred, we could not exclude the mechanical prothrombotic role of CVL in these cases.

Mitchell et al[32] have recently proposed and validated a VTE risk score in children with ALL; they considered the following factors as predictive variables in the risk assessment model: treatment with L-ASP in combination with steroids (PDN or DXM), the presence of CVL, and genetic thrombophilic abnormalities. As the decision to investigate inherited prothrombotic factors and to perform genetic analysis was left to each participating center, we lacked data to evaluate the performance of this score in our cohort.

Three patients died because of the occurrence of VTE. As all deaths occurred within 2 years since the beginning of the AIEOP ALL 2000 study (2/3 deaths in the first 5 mo of the study), we can speculate that a better capacity to handle

the protocol could have led to the absence of deaths in subsequent years. Another issue strongly debated among hemato-oncologists is how to prevent the occurrence of VTE. This aspect is in fact still very controversial. Several reports have attempted to address this issue through a routine assessment of coagulation parameters and the planning of different types of interventions (eg, replacement of coagulation factors such as fibrinogen or antithrombin III, transfusion of fresh frozen plasma, etc.). However, most current ALL protocols do not clearly define whether or how to monitor coagulation tests during the ALL treatment phases including steroids and L-ASP (ie, induction/reinduction); also, replacement indications are rather heterogenous or absent. In the AIEOP-BFM ALL 2000, no indications were given to this purpose. In our study, the laboratory coagulation data entered in the protocol database at the time of VTE onset were analyzed with the aim to evaluate whether coagulation derangement could be a reliable indicator of a higher risk of VTE occurrence. A case-control study was designed specifically to reliably assess the association between coagulation and VTE. This type of study was performed by matching for possible confounding factors and in our opinion is more powerful than the descriptive cross-sectional studies reported in the literature. The results of this comparison showed that severe coagulation derangement was present in $<5\%$ of patients in both groups (cases and controls) and could not be associated with an increased risk of developing VTE. Hence, a direct relationship between the levels of coagulation factors and the frequency of VTE is unlikely. Thus, on the basis of these data, routine monitoring of the coagulation status and therapeutic interventions aimed at reestablishing a normal coagulation profile to prevent the occurrence of VTE cannot be recommended.

The main conclusions derived from this cohort study are that in the population of children with ALL treated according to the AIEOP-BFM ALL 2000 protocol, the rate of VTE was in the expected range (2.4%), that the use of DXM or PDN during the induction phase was not associated with a different risk of VTE (with a nonsignificant increased rate in patients treated with DXM), and that male patients were at a significantly higher risk of developing this complication. VTE led to treatment modifications, relevant morbidity, and even mortality, with 3 patients having died because of the occurrence of a CVT. Overall, this corresponds to approximately 1.5 deaths in every 1000 patients. However, this finding should be matched against the overall mortality rate in the induction and complete remission of a modern and intensive chemotherapy protocol, as reported in the AIEOP-BFM ALL 2000 study (20 for every 1000 patients).

The possibility (and the means) to prevent the onset of VTE is still unclear; the clarification of this clinical aspect remains an urgent need.

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REFERENCES

1. Coebergh JW, Reedijk AM, de Vries E, et al. Leukaemia incidence and survival in children and adolescents in Europe during 1978-1997. Report from the Automated Childhood Cancer Information System project. *Eur J Cancer*. 2006;42:2019–2036.
2. Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood*. 2010;115:3206–3214.
3. Athale UH, Chan AK. Thrombosis in children with acute lymphoblastic leukemia: part I. Epidemiology of thrombosis in children with acute lymphoblastic leukemia. *Thromb Res*. 2003;111:125–131.
4. 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.
5. Athale UH, Chan AK. Thrombosis in children with acute lymphoblastic leukemia Part III. Pathogenesis of thrombosis in children with acute lymphoblastic leukemia: effects of host environment. *Thromb Res*. 2003;111:321–327.
6. Athale UH, Chan AK. Thromboembolic complications in pediatric hematologic malignancies. *Semin Thromb Hemost*. 2007;33:416–426.
7. Payne JH, Vora AJ. Thrombosis and acute lymphoblastic leukaemia. *Br J Haematol*. 2007;138:430–445.

8. Giordano P, Santoro N, Del Vecchio GC, et al. T-immunophenotype is associated with an increased prevalence of thrombosis in children with acute lymphoblastic leukemia. A retrospective study. *Haematologica*. 2003;88:1079–1080.
9. Ratei R, Basso G, Dworzak M, et al. Monitoring treatment response of childhood precursor B-cell acute lymphoblastic leukemia in the AIEOP-BFM-ALL 2000 protocol with multiparameter flow cytometry: predictive impact of early blast reduction on the remission status after induction. *Leukemia*. 2009;23:528–534.
10. Masera G, Conter V, Rizzari C, et al. AIEOP non-B ALL trials. *Int J Ped Hematol Oncol*. 1999;62:102–111.
11. Brown LD, Cai TT, DasGupta A. Interval estimation for a binomial proportion. *Statist Sci*. 2001;16:101–133.
12. Khan S, Dickerman JD. Hereditary thrombophilia. *Thromb J*. 2006;4:15–32.
13. Sutor AH, Mall V, Thomas KB. Bleeding and thrombosis in children with acute lymphoblastic leukaemia, treated according to the ALL-BFM-90 protocol. *Klin Padiatr*. 1999; 211:201–204.
14. Korte W, Feldges A, Baumgartner C, et al. Increased thrombin generation during fibrinogen and platelet recovery as an explanation for hypercoagulability in children with L-asparaginase therapy for ALL or NHL: a preliminary report. *Klin Padiatr*. 1994;206:331–333.
15. Mitchell LG, Andrew M, Hanna K, et al. A prospective cohort study determining the prevalence of thrombotic events in children with acute lymphoblastic leukemia and a central venous line who are treated with L-asparaginase: results of the Prophylactic Antithrombin Replacement in Kids with Acute Lymphoblastic Leukemia Treated with Asparaginase (PARKAA) Study. *Cancer*. 2003;97:508–516.
16. Nowak-Göttl U, Heinecke A, von Kries R, et al. Thrombotic events revisited in children with acute lymphoblastic leukemia: impact of concomitant *Escherichia coli* asparaginase/prednisone administration. *Thromb Res*. 2001;103:165–172.
17. Mauz-Körholz C, Junker R, Göbel U, et al. Prothrombotic risk factors in children with acute lymphoblastic leukemia treated with delayed *E. coli* asparaginase (COALL-92 and 97 protocols). *Thromb Haemost*. 2000;83:840–843.
18. Caruso V, Iacoviello L, Di Castelnuovo A, et al. Thrombotic complications in childhood acute lymphoblastic leukemia: a meta-analysis of 17 prospective studies comprising 1752 pediatric patients. *Blood*. 2006;108:2216–2222.
19. Ribeiro RC, Pui CH. The clinical and biological correlates of coagulopathy in children with acute leukemia. *J Clin Oncol*. 1986;4:1212–1218.
20. Sutor AH, Wulff J, Ritter J, et al. Hemostasis and fibrinolysis in acute lymphoblastic leukemia (ALL) in childhood analysis of life-threatening bleeding. *Klin Padiatr*. 1984;196:166–173.
21. Giordano P, Del Vecchio GC, Santoro N, et al. Thrombin generation in children with acute lymphoblastic leukemia: effect of leukemia immunophenotypic subgroups. *Pediatr Hematol Oncol*. 2000;17:667–672.
22. Monagle P, Michelson AD, Bovill E, et al. Antithrombotic therapy in children. *Chest*. 2001;119:344S–370S.
23. Nowak-Göttl U, Kosch A, Schlegel N. Thromboembolism in newborns, infants and children. *Thromb Haemost*. 2001;86: 464–474.
24. Monagle P, Chan AK, Albisetti M, et al. Fibrinolytic system in adolescents: response to venous occlusion stress tests. *Pediatr Res*. 2003;53:333–337.
25. Appel IM, Hop WC, van Kessel-Bakvis C, et al. L-Asparaginase and the effect of age on coagulation and fibrinolysis in childhood acute lymphoblastic leukemia. *Thromb Haemost*. 2008;100:330–337.
26. Athale UH, Siciliano S, Thabane L, et al. Epidemiology and clinical risk factors predisposing to thromboembolism in children with cancer. *Pediatr Blood Cancer*. 2008;51:792–797.
27. Nowak-Göttl U, Wermes C, Junker R, et al. Prospective evaluation of the thrombotic risk in children with acute lymphoblastic leukemia carrying the MTHFR TT 677 genotype, the prothrombin G20210A variant, and further prothrombotic risk factors. *Blood*. 1999;93:1595–1599.
28. Pui CH, Chesney CM, Weed J, et al. Altered von Willebrand factor molecule in children with thrombosis following asparaginase-prednisone-vincristine therapy for leukemia. *J Clin Oncol*. 1985;3:1266–1272.
29. Priest JR, Ramsay NK, Steinherz PG, et al. A syndrome of thrombosis and hemorrhage complicating L-asparaginase therapy for childhood acute lymphoblastic leukemia. *J Pediatr*. 1982;100:984–989.
30. Santoro N, Giordano P, Del Vecchio GC, et al. Ischemic stroke in children treated for acute lymphoblastic leukemia: a retrospective study. *J Pediatr Hematol Oncol*. 2005;27:153–157.
31. Nowak-Göttl U, Ahlke E, Fleischhack G, et al. Thromboembolic events in children with acute lymphoblastic leukemia (BFM protocols): prednisone versus dexamethasone administration. *Blood*. 2003;101:2529–2533.
32. Mitchell L, Lambers M, Flege S, et al. Validation of a predictive model for identifying an increased risk for thromboembolism in children with acute lymphoblastic leukemia: results of a multicenter cohort study. *Blood*. 2010;115: 4999–5004.

