Thrombotic complications in childhood acute lymphoblastic leukemia: a meta-analysis of 17 prospective studies comprising 1752 pediatric patients

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The risk of thrombosis in children with acute lymphoblastic leukemia (ALL) reportedly ranges between 1% and 37%. Epidemiologic studies have usually been hampered by small numbers, making accurate estimates of thrombosis risk in ALL patients very difficult. The aim of this study was to better estimate the frequency of this complication and to define how the disease, its treatment, and the host contribute to its occurrence. We made an attempt to combine and analyze all published data on the association between pediatric ALL and thrombosis, by using a meta-analytic method. The rate of thrombosis in 1752 children from 17 prospective studies was 5.2% (95% CI: 4.2-6.4). The risk varies depending on several factors. Most of the events occurred during the induction phase of therapy. Lower doses of asparaginase (ASP) for long periods were associated with the highest incidence of thrombosis, as were anthracyclines and prednisone (instead of dexamethasone). The presence of central lines and of thrombophilic genetic abnormalities also appeared to be frequently associated with thrombosis. In conclusion, the overall thrombotic risk in ALL children was significant, and the subgroup analysis was able to identify high-risk individuals, a finding that will hopefully guide future prospective studies aimed at decreasing this risk. (Blood. 2006;108:2216-2222)

Materials and methods

Meta-analysis

In the PubMed database, all available articles were searched using the following keywords: “acute lymphoblastic leukemia/leukemia” alone and combined with thrombosis, “thrombotic events,” hypercoagulability, and coagulation. Subsequently, cross-references were searched for.

In addition to studies specifically designed to evaluate thrombosis occurrence in ALL patients, articles dealing with treatment protocols describing thrombosis episodes as adverse events were included, as well as papers reporting laboratory findings on hypercoagulability associated with clinical events.

Only English-written articles were included, if published after 1970, when ASP started to be regularly used.

“Symptomatic” thrombotic events were the end points used for this analysis. These were defined either as “symptomatic thrombotic event,” or “symptomatic thrombosis,” “symptomatic venous thrombosis,” or merely “thrombosis/thrombotic event,” but followed by description of clinical symptoms. All events were confirmed by objective methods. Studies in which the definition of events was unclear or unspecific (such as “coagulopathy [thrombosis OR clinical bleeding]”) or “clinical or biologic coagulation abnormality requiring a modification of chemotherapy or supportive care”) were excluded.

Patients included in different arms of observational comparative studies or randomized clinical trials were considered as belonging to different “populations.”

Extracted data were as follows: site of thrombotic event, whether it occurred during or following the induction phase of therapy, characteristics of the study population (mean age, risk stratification, ALL subtype, use of...
central venous catheters [CVCs], prothrombotic genetic defects when studied), and treatment protocol (drugs included, type and dosage of ASP, type and moment of administration of steroids, and antithrombotic measures when applied). Approval was obtained from the Catholic University at Campobasso institutional review board for these studies. Informed consent was obtained according to the Declaration of Helsinki.

Selection of articles
From a total of 100 articles retrieved, 67 were excluded for one of the following reasons: case reports without reference to the population at risk (12),6-17 no data about the incidence of thrombosis (20),18-37 reviews (7),2,4,38-41 duplicated data (4),2,45 no clear definition of the end points (2),46,47 and studies on adult patients (cut-off age from 14 to 20 years) (22).48-69

From the PARKAA study,44,45,70 only symptomatic events were taken into account.

The final model comprised 17 studies (24 populations), including 1752 patients and 91 events. The mean age of this pooled population was 5.5 years.

Description of the events
Of 91 events, 49 occurred in the central nervous system (CNS), while 39 were venous thrombosis in other sites. Twenty-six cerebral events were clearly defined by the authors as being of venous origin. The remaining events were less clearly defined (Table 2).

Table 2. Sites of thrombosis

<table>
<thead>
<tr>
<th>Site of thrombosis</th>
<th>No. of events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td>49 (53.8)</td>
</tr>
<tr>
<td>Cerebral venous thrombosis</td>
<td>26 (28.6)</td>
</tr>
<tr>
<td>Cerebral thrombosis (nonspecified)</td>
<td>5 (5.5)</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>9 (9.9)</td>
</tr>
<tr>
<td>Stroke</td>
<td>9 (9.9)</td>
</tr>
<tr>
<td>Non-CNS venous thrombosis</td>
<td>39 (42.8)</td>
</tr>
<tr>
<td>Nonspecified DVT</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td>DVT-lower limbs</td>
<td>7 (7.7)</td>
</tr>
<tr>
<td>DVT-upper limbs + CVC-associated thrombosis</td>
<td>25 (27.5)</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Right atrium</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Portal thrombosis</td>
<td>0</td>
</tr>
<tr>
<td>Superficial thrombosis</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Nonspecified site of thrombosis</td>
<td>3 (3.3)</td>
</tr>
</tbody>
</table>

Subgroups analysis
Thrombosis rate was separately estimated for different subgroups of studies. These were created taking into account phases of therapy, differences in treatment, year of publication of the study, and presence of genetic prothrombotic defects.

Phases of therapy. Three distinct IRs of thrombosis were estimated.

The “global” IR represents the risk of a thrombotic event during the entire duration of the treatment (from diagnosis of ALL to the end of the maintenance phase). All selected studies were considered in this estimation. Many studies described only the induction phase of ALL treatment, whereas a small amount evaluated only postremission phases, such as consolidation or maintenance. These articles were pooled separately to estimate individual IRs for each of these phases (defined as “induction” and “postinduction”). Some authors observed the patients during the whole length of treatment but specified when the event occurred, allowing us to use their articles for the estimation of both global and phase-specific IRs of thrombosis.

Differences in treatment. The differences were evaluated by considering the following: type and dose of ASP, type of steroids, concomitant use of ASF and steroids, use of anthracyclines, and anti-thrombin III supplementation.

Year of publication of the study. The studies were subgrouped according to publication before or in 1989, between 1990 and 1999, and in or after 2000.

Presence of genetic prothrombotic defects. Studies evaluating the presence of genetic prothrombotic risk factors in the host were included in a separate meta-analysis in order to evaluate the risk of thrombosis in ALL patients with thrombophilia.

Statistical analysis
Pooled IRs were calculated using an exact method, as proposed in Martin and Austin.103 Briefly, this approach used exact maximum likelihood binomial distribution for calculating pooled rates and 95% confidence intervals; 0.5 was not added to numbers of events in studies with 0 events because the method accounts for sparseness of individual studies. Homogeneity across studies was tested using the Breslow-Day test. The method provides stratum-specific estimates and tests of differences across subgroups.

Results
Global meta-analysis
When considering all prospective studies, the global IR of symptomatic thrombosis was 5.2% (95% CI: 4.2-6.4) (homogeneity test: \( P < .001 \)).

The global IRs of CNS and non-CNS events were 2.9% (95% CI: 2.2-3.8) and 2.3% (95% CI: 1.7-3.2), respectively.

Subgroup analyses
Phases of therapy. Thirteen studies reported events occurring specifically during the induction phase of ALL treatment. A total of...
61 events were observed in 1280 patients, corresponding to an IR of 4.8% (95% CI: 3.7-6.0).

Twelve events occurred in later phases of treatment (consolidation and maintenance) in 609 patients (7 studies); the thrombosis IR was 2.0% (95% CI: 1.1-3.3); P = .004.

**Influence of therapy on the incidence of thrombosis during induction. Type of Asparaginase.** No thrombotic event was observed in the small group of patients (one study with 10 patients) treated with Erwinase-ASP (IR: 0%; 95% CI: 0-30) instead of *Escherichia coli*–ASP (IR: 4.8%; 95% CI: 3.7-6.1; P = .49).

Separate IRs of thrombosis were estimated for studies mentioning the brand of *E coli*–ASP preparation. The population receiving *E coli*–ASP Medac (Kyowa Hakko, Kyogo, Japan) showed more events (patients: 618; events: 44; IR: 8.7%; 95% CI: 6.4-11.5) than the one receiving *E coli*–ASP Crasnitin (Bayer, Leverkusen, Germany) (patients: 30; events: 0; IR: 0%; 95% CI: 0-10.0; P = .14). In the studies where the preparation of *E coli*–ASP was unknown (patients: 632; events: 17), the IR of thrombosis was 2.4% (95% CI: 1.5-3.7), significantly lower than that observed in the *E coli* Medac group (P = .001).

**Doses of Asparaginase.** The total dose of ASP received (calculated as daily dose × days of treatment) was first evaluated. Then, daily doses and length of administration were independently used to constitute patient subgroups (Table 3).

No difference in thrombosis incidence was found among 3 groups receiving increasing total doses of ASP (20 000 to 59 000 U/m²; 60 000 to 79 000 U/m²; and ≥ 80 000 U/m²).

Thrombotic events were significantly more numerous in the group receiving lower doses of ASP (< 6000 U/m²; generally 5000 or 6000 U/m²) versus 10 000 U/m² or more (generally 10 000 or 25 000 U/m²).

Patients who received ASP for more than 9 days had a higher incidence of thrombosis. Daily doses of ASP and length of therapy were combined in 4 groups. Although a longer administration of ASP was associated with a higher incidence of thrombosis, a lower dosage of this drug increased the risk within each group.

When evaluating only studies using *E coli*–ASP Medac, IR of thrombosis did not change with different daily dosages of the drug.

No significant difference was observed in the incidence of thrombotic events in 1134 patients given ASP 2 to 3 times weekly, compared with 103 patients receiving the drug on consecutive days.

**Type of steroids.** In one prospective study, dexamethasone was used instead of prednisone during induction. This included 56 patients in whom 3 thrombotic events were recorded (IR: 2.0%; 95% CI: 0.3-8.8), a rate not significantly lower than that observed in patients receiving prednisone (4.9%; 95% CI: 3.8-6.3) (P = .30).

Only 26 of 543 patients with available data about treatment in postinduction phases were treated with prednisone instead of dexamethasone. Three of 12 events described in postinduction phases occurred in the prednisone group, accounting for a significant increase in the risk of thrombosis during this period (12.2%; 95% CI: 2.9-31.4 vs 1.6%; 95% CI: 0.8-2.8; P = .001).

**Concomitant administration of ASP and steroids.** In the 2 prospective studies where ASP and steroids were not given concomitantly during induction (10% of the total population), only one event was observed. The corresponding IR of 4.2% (95% CI: 1.7-8.5) was not significantly different from that calculated in the larger group of patients receiving concomitant administration of ASP and steroids (5.3%; 95% CI: 4.3-6.5) (P = .55).

**Anthracyclines.** The IR was significantly lower in 495 patients from 3 studies not receiving anthracyclines as part of the induction treatment (2.7%; 95% CI: 1.5-4.27) than in the remaining 785 patients who received daunorubicin, doxorubicin, or idarubicin (6.1%; 95% CI: 4.6-8.0; P = .005).

No difference was observed in the risk of thrombosis when comparing different types of anthracyclines.

**Prophylaxis with anti–thrombin III.** As only one prospective study compared prophylaxis with anti–thrombin III against no supplementation, no analysis of this variable could be performed.

**Effect of publication period.** The IR of thrombosis was found to be significantly higher when pooling more recent versus older publications (Figure 1).

The positive association between lower doses of ASP and thrombosis was still observed when assessing each decade separately (data not shown).

**Presence of prothrombotic genetic defects.** Prothrombotic genetic defects were investigated in 557 children (5 studies). Thirty-one events were observed in 113 patients affected by at least one prothrombotic genetic risk factor (factor V 1691G>A mutation, prothrombin 20210G>A variant, TT677 methylenetetrahydrofolate reductase [MTHFR] genotype, deficiency of protein C, protein S, antithrombin, elevated lipoprotein [a]). As only one study measured antiphospholipid antibodies (APLAs), this acquired prothrombotic condition was not included in the analysis.

### Table 3. IR of thrombosis according to different doses of L-Asparaginase

<table>
<thead>
<tr>
<th>Total dose</th>
<th>Populations</th>
<th>N</th>
<th>Events</th>
<th>IR, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 000 to 59 000 U/m²</td>
<td>7</td>
<td>202</td>
<td>8</td>
<td>3.9 (1.8-7.5)</td>
</tr>
<tr>
<td>60 000 to 79 000 U/m²</td>
<td>2</td>
<td>760</td>
<td>41</td>
<td>5.4 (3.9-7.2)</td>
</tr>
<tr>
<td>80 000 U/m² or higher</td>
<td>8</td>
<td>318</td>
<td>12</td>
<td>3.8 (2.0-6.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Daily dose</th>
<th>N</th>
<th>Events</th>
<th>IR, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 000 U/m² or less</td>
<td>7</td>
<td>476</td>
<td>38</td>
</tr>
<tr>
<td>10 000 U/m² or higher</td>
<td>10</td>
<td>804</td>
<td>23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total days</th>
<th>N</th>
<th>Events</th>
<th>IR, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fewer than 9 days</td>
<td>14</td>
<td>945</td>
<td>29</td>
</tr>
<tr>
<td>9 or more days</td>
<td>3</td>
<td>335</td>
<td>32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interaction between ASP daily dose and length of therapy</th>
<th>Populations</th>
<th>N</th>
<th>Events</th>
<th>IR, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fewer than 9 days, 6 000 U/m² or less</td>
<td>4</td>
<td>141</td>
<td>6</td>
<td>4.4 (1.7-8.8)</td>
</tr>
<tr>
<td>Fewer than 9 days, 10 000 U/m² or higher</td>
<td>10</td>
<td>804</td>
<td>23</td>
<td>2.9 (1.8-4.2)</td>
</tr>
<tr>
<td>9 or more days, 6 000 U/m² or less</td>
<td>3</td>
<td>335</td>
<td>32</td>
<td>9.6 (6.6-13.3)</td>
</tr>
<tr>
<td>9 or more days, 10 000 U/m² or higher</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Heterogeneity across groups, P**

| .46 |
| <.001 |
| <.001 |
| <.001 |
The prevalence of genetic prothrombotic abnormalities was similar to that usually found in the general pediatric population. Pooling the 5 studies, the thrombotic risk in ALL patients with thrombophilia increased approximately 8-fold (relative risk [RR]: 8.5; 95% CI: 4.4-17.4) (Figure 2).

Discussion

Thrombosis is uncommon in children, but it may occur in some pathologic conditions, such as ALL. The prevalence and the pathogenesis of thrombosis associated with ALL are obscure. The primary disease itself can activate blood coagulation via procoagulant substances or by impairment of fibrinolytic or anticoagulant pathways. Additionally, chemotherapy and prothrombotic risk factors of the host might play a contributory role. Epidemiologic studies of pediatric thrombosis in ALL have been greatly hampered by small numbers, making estimates of thrombosis risk in this condition very difficult.

The aim of this study was to better estimate the frequency of this association by quantitatively pooling and analyzing all available data and to define how the primary disease, its treatment, and the host contribute to its occurrence. Although we tried to minimize possible biases by accurate sensitivity analyses, this meta-analysis has some limitations due to the great heterogeneity in study designs, ALL populations, and treatment protocols. However, this is one of the first attempts to quantitatively combine and analyze existing data on thrombosis and ALL in a pediatric population, and may represent an important basis for generating hypotheses to be tested by future studies.

Occurrence of different thrombotic events in ALL

The estimated occurrence of thrombosis during treatment (from diagnosis of ALL to the end of the maintenance phase) was 5.2%. This is not a trivial figure, since the estimated annual incidence of venous thrombosis in the pediatric population is about 1 per 100,000 children. Thrombotic events mainly occurred in the central nervous system and in the upper limbs, the latter being frequently associated with CVCs.

Cerebral events are critical complications of ALL treatment, while neurologic manifestations can be secondary to hemorrhagic or ischemic episodes. In addition, drug-related neurologic adverse events and CNS involvement of the primary disease sometimes mimic stroke syndromes. Of the 49 CNS events described in our population, only 26 (28.6% of total) were described by the authors as “cerebral venous thrombosis.” Others were described as “cerebral thrombosis,” without mentioning whether they were arterial or venous. Other definitions, such as “cerebral infarction” and “stroke” do not allow hemorrhagic events to be ruled out. In fact, one of these events was described only as “stroke-like episode.” These rather vague definitions did not permit an accurate estimate of ischemic CNS events rate to be made. Although cerebral venous thrombosis is a rare condition, it is frequently associated with hematologic disorders.

Peripheral arterial thrombotic events were not reported, suggesting that the prothrombotic imbalance could predispose ALL patients mainly to venous thrombosis. This finding is not surprising, as venous and arterial thrombotic diseases, while sharing some common mechanisms, differ for most risk factors.

Effect of ALL treatment on the occurrence of thrombosis

Most of the thrombotic events occurred during the induction phase. This is not unexpected, as treatment is more intense during this initial phase, and, more importantly, the disease is still active at the beginning of therapy, thus yielding a large lymphoblast burden undergoing cytolysis. Patients receiving postinduction treatment have a more stable disease; as a consequence, the intensity of the treatment and cell lysis are less pronounced.

Use of dexamethasone instead of prednisone was reportedly associated with a lower incidence of thrombotic events. In this meta-analysis, the population receiving prednisone during induction had more thrombotic events than those receiving dexamethasone; however, this difference was not statistically significant, probably because of the small number of patients receiving the latter steroid. In accordance, during postinduction phases, most of thrombotic events took place in the small group of patients receiving prednisone instead of dexamethasone. These findings, along with the data on the reduction in thrombosis rate when ASP and prednisone were administered separately, are important therapeutic issues that should be investigated in larger clinical studies.
Lower doses of ASP given for a longer period of time increase the risk of thrombosis

ASP is an essential drug for the treatment of ALL. However, it might impair the hematic system by reducing the synthesis of both coagulation factors and inhibitors, as a consequence of asparaginase depletion. It has been previously observed in laboratory studies that Erwinase-ASP and some E. coli-ASP preparations, such as L-ASP Crasinitin, only partially deplete the asparagine pool and are associated with fewer thrombotic complications, but also with a reduced antineoplastic efficacy. As the degree of coagulation abnormalities correlates with ASP activity, ASP-related thrombotic risk appears to be a “price” to be paid to guarantee an effective treatment to ALL patients.

In this meta-analysis, we examined whether the influence of ASP on thrombosis could differ according to different modalities of administration. In the last years, the tendency was to decrease the dose (from 10,000 to 5000-6000 U/m²), to reduce the length of therapy (from 14 to 7-9 days), and to increase the interval between doses. The finding that patients receiving ASP for longer periods experienced more thrombotic events was not unexpected, but the association between lower doses of ASP and higher rates of thrombosis is a novel observation. The latter association was not found when only patients who received L-ASP Medac were evaluated. Unfortunately, few studies described the type of preparation of E. coli–ASP, and in the larger group of patients in which the brand of E. coli–ASP was unknown, the inverse relationship between ASP dose and thrombotic events was significant.

Furthermore, the progressive increase of thrombosis risk in the several last years might be partly due to more recent introduction of aggressive therapy along with lower doses of ASP. The improvement in diagnostic tools in recent years might also explain the larger number of thrombotic events detected in the latest publications.

Thrombophilia and CVCs: should we screen to prevent thrombosis?

Contradictory results had been reported by studies checking for thrombophilia in ALL patients with thrombotic events. While some failed to show any association between genetic prothrombotic defects and increased risk of thrombosis, others found that thrombophilia represents an important additional risk factor in this population. Meta-analysis of these 5 studies showed that the presence of at least one genetic prothrombotic factor was associated with an 8-fold increase in the risk of thrombosis in children with ALL. This figure, although impressive, cannot disregard the role of hereditary disorders of coagulation abnormalities correlates with ASP activity. ASP-related thrombotic risk appears to be a “price” to be paid to guarantee an effective treatment to ALL patients.

In conclusion, the induction treatment of ALL appears to predispose pediatric patients to thrombotic complications due to a combination of factors linked to the disease itself, to its treatment, to the genetic background of the host, and to some acquired conditions. The difficulty in eliminating all possible confounders in this meta-analysis does not allow for a definite statement that lower doses of ASP increase the risk of thrombosis. Detailed information on the kinetics of ASP in children is lacking; therefore, our findings could encourage studies aimed at determining whether ASP therapy could be improved in ALL pediatric patients by a careful individualized risk-efficacy approach. Data on the reduction in thrombotic events rate when ASP and prednisone were administered separately and when dexamethasone instead of prednisone is given are significant and should be supported by larger clinical studies. In the meantime, systematic screening for prothrombotic risk factors should be encouraged, in the setting of well-designed prospective clinical studies, as a strategy to identify those patients at higher risk of thrombosis, in whom prophylactic approaches presently lacking could be developed.

Acknowledgment

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References


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