Haemostatic alterations induced by treatment with asparaginases and clinical consequences

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**Summary**

The benefit of asparaginase for treating acute lymphoid leukaemia (ALL) has been well established. Native asparaginase derives from *Escherichia coli* (colaspase) or *Erwinia chrysanthemi* (crisantaspase); in a third preparation, colaspase is pegylated. Depletion of asparagine leads to decreased synthesis of procoagulant, anticoagulant, and fibrinolytic proteins, with resultant hypercoagulability and greater risk of venous thromboembolism (VTE). Colaspase and crisantaspase are not dose-equivalent, with crisantaspase displaying haemostatic toxicity only at dosages much higher and administered more frequently than those of colaspase. Cerebral venous thrombosis and pulmonary embolism are two life-endangering manifestations that occur during treatment with asparaginase particularly in children and in adults with ALL, respectively. Approximately one-third of VTEs are located in the upper extremities and are central venous line-related. Other risk factors are longer duration of asparaginase treatment and concomitant use of prednisone, anticyclicines, and oral contraceptives. The risk associated with inherited thrombophilia is uncertain but is clearly enhanced by other risk factors or by the use of prednisone. VTE prevention with fresh frozen plasma is not recommended; the efficacy of antithrombin (AT) concentrates has occasionally been reported, but these reports should be confirmed by proper studies, and AT should not be routinely employed. Therapeutic or prophylactic heparin doses are only partially effective, and direct thrombin or factor Xa inhibitors could play significant roles in the near future.

**Keywords**
Asparaginase, hypercoagulability, thrombosis, antithrombin concentrate, heparin

**Introduction**

In 1953, Kidd first described an activity-inducing regression of transplanted lymphomas in mice and rats that was present in the serum of guinea pigs but not in that of horses or rabbits (1, 2). In 1961, this antilymphoma activity was found to be due to asparaginase (3). Moreover, some experimental neoplasms have been found to require asparagine to support their growth in tissue culture (4, 5). Finally, asparaginase isolated from *Escherichia coli* has been demonstrated to have antitumour activity similar to that of guinea pig serum (6–8), so the production of large quantities of the enzyme for preclinical and clinical investigations has been possible (9).

Asparagine is an amino acid required by cells for the production of proteins; it can either be produced within a cell through the enzyme called asparagine-synthetase or be absorbed into the cell from the outside. The enzyme asparaginase catalyses the hydrolysis of asparagine to L-aspartic acid and ammonia, resulting in depletion of the circulating pool of asparagine in the plasma (10–15) and, in part, in the cerebrospinal fluid (CSF) (16), forcing cells to rely completely on what they can produce on their own. Normal cells do not require nearly as much asparagine to survive, and they are able to produce all of the asparagine they need internally. Tumour cells, in contrast, require enormous amounts of asparagine to keep up with their rapid, malignant growth, but in some cases, they lack asparagine-synthetase (i.e. they require an exogenous source of asparagine for protein synthesis). Human lymphoblasts require significant amounts of asparagine for protein synthesis and proliferation, but they have low levels of asparagine-synthetase, so they are dependent on extracellular asparagine and are deprived of it by the administration of asparaginase (17). Moreover, asparaginase catalyses the hydrolysis of glutamine to glutamic acid, resulting in the depletion of circulating levels of this amino acid (18). Glutamine acts as a nitrogen donor for a number of enzymes involved in the de novo synthesis of purine and pyrimidine nucleotides (19) and as the amide donor for the synthesis of asparagine. Therefore, depletion of asparagine and glutamine by asparaginase leads to cell-cycle arrest and apoptotic cell death of leukaemia cells (20).

Extensive clinical data have supported the use of asparaginase therapy in paediatric acute lymphoblastic leukaemia (ALL), and the benefit of intensive asparaginase treatment, compared with less intensive regimens, has been demonstrated (21, 22). Asparaginase used in the clinic is available in three preparations: two unmodi-
Asparaginase-related complications

De Stefano et al.

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Effects of asparaginase on the coagulation system

The impairment of protein synthesis due to depletion of asparagine by asparaginase leads to decreases in albumin (26), insulin (27), thyroxine-binding globulin (28), complement components (29), and almost all of the factors involved in coagulation and fibrinolysis (30–82) (Table 2). The content of asparagine in coagulation proteins ranges between 2.2% and 6.8% (68), but there is no relationship between asparagine content and the magnitude of the effect. The causes of the decreases in coagulation protein levels are possibly confounded by the occurrence of the low-grade intravascular coagulation in patients with ALL. However, in the first studies investigating this issue, decay of fibrinogen labelled with $^{125}$I and injected into patients receiving asparaginase was not accelerated, providing evidence against the intravascular consumption of fibrinogen (31, 83).

In humans, the circulating half-lives of the asparaginase enzymes from *E. coli* and *Erwinia chrysanthemi* vary widely (84, 85) (Table 1). Moreover, the half-lives differ not only between *E. coli* strains type A and type B but also among different commercial *E. coli* preparations (88–89). A lower clearance and a longer half-life result in higher levels of asparaginase activity and a prolonged duration of asparagine depletion. These effects possibly induce prolonged inhibition of protein synthesis, as well as undesired effects on other amino acids and proteins; in summary, they can cause a higher rate of side effects even with the same dose (74), in part explaining some inconsistencies in the reports addressing the levels of circulating proteins involved in haemostasis (90) (Table 2). Another confounding factor can be the concomitant use of steroids. Sutor et al. (81) reported that fibrinogen decreased during prednisone therapy, and antithrombin (AT) and protein C (PC) increased, whereas plasminogen remained unchanged. During prednisone and colaspase therapy, fibrinogen continued to decrease, and plasminogen, AT, and PC also decreased. In another study of ALL children treated with steroids and colaspase, significantly lower values of fibrinogen, AT, plasminogen, and free protein S (PS) antigen were found in the group receiving prednisone, compared with that receiving dexamethasone. Von Willebrand factor (VWF) levels were significantly higher in children treated with dexamethasone. No difference was found for PC (80).

Effects on procoagulant factors

Asparaginase reduces levels of the coagulation proteins, as evidenced by the decreases in fibrinogen, factor II, factor IX, factor V, factor XIII, and factor X (86–87) (Table 1). Moreover, the half-lives differ not only between *E. coli* strains type A and type B but also among different commercial *E. coli* preparations (88–89). A lower clearance and a longer half-life result in higher levels of asparaginase activity and a prolonged duration of asparagine depletion. These effects possibly induce prolonged inhibition of protein synthesis, as well as undesired effects on other amino acids and proteins; in summary, they can cause a higher rate of side effects even with the same dose (74), in part explaining some inconsistencies in the reports addressing the levels of circulating proteins involved in haemostasis (90) (Table 2). Another confounding factor can be the concomitant use of steroids. Sutor et al. (81) reported that fibrinogen decreased during prednisone therapy, and antithrombin (AT) and protein C (PC) increased, whereas plasminogen remained unchanged. During prednisone and colaspase therapy, fibrinogen continued to decrease, and plasminogen, AT, and PC also decreased. In another study of ALL children treated with steroids and colaspase, significantly lower values of fibrinogen, AT, plasminogen, and free protein S (PS) antigen were found in the group receiving prednisone, compared with that receiving dexamethasone. Von Willebrand factor (VWF) levels were significantly higher in children treated with dexamethasone. No difference was found for PC (80).

### Table 1: Properties of therapeutic asparaginases

| Table 2: Properties of therapeutic asparaginases. Asp, L-Gln, and pl refer to asparaginase, L-glutaminase, and isoelectric point, respectively (modified from [23]). |

<table>
<thead>
<tr>
<th>Asparaginase</th>
<th><em>E. coli</em> native (colaspase)</th>
<th><em>E. coli</em> PEG (pegaspase)</th>
<th><em>Erwinia chrysanthemi</em> (crisantaspase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity* (IU/mg protein)</td>
<td>280–400</td>
<td>280–400</td>
<td>650–700</td>
</tr>
<tr>
<td>Ratio maximal activity L-Gln/Asp</td>
<td>0.03</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>141,000</td>
<td>145,000</td>
<td>138,000</td>
</tr>
<tr>
<td>PI</td>
<td>5.0</td>
<td>5.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Half-life (days)</td>
<td>0.6–1.0</td>
<td>6.0–7.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Duration of asparagine depletion†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In native patients</td>
<td>7–10 days</td>
<td>14–35 days</td>
<td>5–7 days</td>
</tr>
<tr>
<td>In exposed patients‡</td>
<td>2–3 days</td>
<td>5–14 days</td>
<td>1–2 days</td>
</tr>
<tr>
<td>In hypersensitive patients</td>
<td>0 days</td>
<td>1–3 days</td>
<td>0 days</td>
</tr>
</tbody>
</table>

*One international unit hydrolyses 1 micromole of asparagine per minute. †After a single dose of 25,000 IU/m² of native colaspase or crisantaspase or 2,500 IU/m² of pegaspase in front-line native, asparaginase-exposed, or clinically hypersensitive patients. ‡Prior therapy with native *E. coli* asparaginase without a history of clinical hypersensitivity.

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factor X, and prothrombin (Table 2). These decreases suggest that there might be global inhibition of the production of coagulant proteins in the liver.

However, asparagine residues are critical to the structures of clotting factors. Hydroxylated aspartic acid and asparagine residues were first identified in the vitamin K–dependent clotting factors: hydroxylated aspartic acid in PC, PS, factors IX and X, and in protein Z; and hydroxylated asparagine in PS. In human factor IX, the hydroxylation is partial. Hydroxylated aspartic acid and asparagine are formed by postribosomal hydroxylation of aspartic acid and asparagine, respectively, and have so far been identified only in EGF-like domains (92).

Results concerning factor VIII and VWF levels have been contrasting: in one study, VWF antigen levels significantly increased with the number of asparaginase infusions during induction (82), likely mirroring endothelial perturbation due either to disease or to asparaginase administration, as recently reviewed (93).

**Effects on anticoagulant factors**

The two main regulatory pathways of coagulation consist of the inhibition of serine proteases by AT and that of non-enzymatic cofactors VIIIa and Va by activated PC and its cofactor PS (94, 95).

One of the main effects of asparaginase treatment is a reduction in AT levels. AT, a member of the serine protease inhibitor (serpin) superfamily synthesised in the liver, regulates coagulation by forming a 1:1 covalent complex with thrombin, factor Xa and other activated procoagulants, including factors XIIa, Xla, IXa and VIIa. Heterozygous congenital deficiency is associated with severe thrombophilia, having a relative risk of venous thromboembolism (VTE) 10-fold increased relative to the general population, and the homozygous state is believed to produce embryonic lethality (96).

In general, the effects of asparaginase on AT synthesis seem greater than those observed for other clotting proteins (Table 2 and Figure 1). The preferential decrease in plasma concentrations of AT could reflect both impaired hepatic protein synthesis and increased consumption due to increased endogenous generation of thrombin. Intracellular AT from asparaginase-treated cells differed in isoelectric point, suggesting that asparaginase could interfere with glycosylation and the folding of the molecule (97). Hepatocarcinoma (HepG2) cells treated with asparaginase showed a marked reduction in AT secretion but no reduction in AT mRNA levels, suggesting interference at a post-transcriptional stage. Moreover, in HepG2 cells, intracellular accumulation of AT aggregates was detected, and a similar pattern was observed in mice, with retention and aggregation of the protein within the lumen of dilated rough endoplasmic reticulum cisterns (98). These findings were more evident in patients and in cell and mouse models exposed to asparaginase alone or in combination with...
prednisone, whereas the combination of asparaginase with dexamethasone ameliorated both the deficiency and intracellular retention of AT, in association with increased expression of heat shock proteins and endoplasmic reticulum-chaperons (99).

Inhibition of coagulation is further hampered by the effects of asparaginase on other factors, such as heparin cofactor II, PC and PS (Table 2 and Figure 1).

### Effects on the fibrinolytic factors

Downregulation of plasminogen and α-2-antiplasmin, together with an increase in plasminogen activator inhibitor (PAI)-1, has been consistently reported (Table 2 and Figure 1).

In general, the elevation of PAI-1 and the marked decrease in plasminogen levels suggest that, during the course of asparaginase treatment, the coagulation-fibrinolysis balance is shifted towards promotion of fibrin formation and deposition. Indeed, no studies have been conducted using a global test, such as the clot lysis time.

### Effects on the platelet function

A direct platelet agonist effect of asparaginase has been reported in vitro (100) and in vivo to increase the response to ADP (101). In contrast, an isolated abnormality of platelet aggregation in response to collagen was found in ALL patients during the course of therapy, with normalisation of platelet aggregation in response to collagen following the discontinuation of asparaginase (102). However, aggregation induced by ADP, epinephrine, and collagen did not change significantly in platelets from patients treated with asparaginase in respect to the basal values (57) or to control platelets (100), neither Platelet Factor 4 was increased following administration of asparaginase (57).

### Markers of hypercoagulability during asparaginase treatment

In some studies in ALL patients at presentation, plasma concentrations of markers of in vivo thrombin activity (e.g. prothrombin-activation peptide 1+2 [F1+2], thrombin-antithrombin [TAT] complexes) have been found to be increased, and neither asparaginase alone nor combination chemotherapy, with or without asparaginase, significantly altered such values (71, 103). In other studies, the plasma peptides that reflect the activation of coagulation (D-dimer, fibrinopeptide A [FPA], F1+2, TAT complexes) were higher than in normal controls prior to asparaginase administration, but they further increased during treatment (61, 67) (Table 2). Interestingly, this latter phenomenon was not present in patients receiving AT supplementation (69, 104–106); these observations were not confirmed in a large, randomised study, in which no differences in the concentrations of TAT complexes or...

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**Figure 1:** Main putative mechanisms associated with asparaginase-induced thrombosis, indicated by the gray arrows and involving the natural inhibitors of coagulation (decrease in antithrombin levels and, at a lesser extent, in proteins C and S levels) and fibrinolysis (decrease in plasminogen and alpha-2-antiplasmin levels and increase in PAI levels). APC: activated protein C; AT: antithrombin; α2 AP: alpha-2-antiplasmin; F: factor; PAI: plasminogen activator inhibitor; PC: protein C; PS: protein S; TF: tissue factor; TFPI: tissue factor pathway inhibitor; TM: thrombomodulin; t-PA: tissue-plasminogen activator.
F1+2 were observed in patients with and without regular AT substitution (107).

Thrombin generation, globally measured by calibrated automated thrombography, in children with ALL treated with asparaginase was found to be significantly higher during the induction phase compared with reinduction, and it was not substantially affected by hypofibrinogenaemia and/or AT deficiency (108).

Effects on the coagulation system employing different asparaginase sources

Crisantaspase has been claimed to alter the coagulation system less severely than colaspase does (109). One small-sized study (10 patients per arm) comparing the effects on haemostasis of colaspase and crisantaspase (10, 000 U/m² on days 8 to 14 of the induction protocol) showed that the two formulations had similar effects on the decreases in AT, PC, and plasminogen levels, and no difference was noticed in the TAT complexes. In contrast the fibrinogen level was more severely and more frequently decreased by colaspase (65). Another study with the same design, size, and dosage of asparaginase but a different schedule of administration (from day 19 to 40 of induction every three days for a total of eight doses) showed a significant decrease in plasminogen and α2-antiplasmin levels in the patients treated with colaspase but not in those treated with crisantaspase (103). The available data are summarised in Table 2.

The European Organisation for Research and Treatment of Cancer–Children's Leukemia Group (EORTC-CLG) 58881 trial randomised 700 children with ALL or lymphoblastic lymphoma to either colaspase or crisantaspase at the same dosage of 10,000 U/m² twice weekly. Coagulation abnormalities were more frequent in the colaspase arm than in the crisantaspase arm of the study (30.2% vs 11.9%, p<0.0001). The incidence of other toxicities was not significantly different. In the crisantaspase arm, more patients failed to achieve complete remission (4.9% vs 2.0%, p=0.03), and the relapse rate was higher, leading to shorter event-free survival (hazard ratio [HR], 1.59; 95% confidence interval [CI], 1.23–2.06) (110).

This finding was due to the difference in pharmacokinetics of the two asparaginase preparations, with crisantaspase producing significantly lower serum asparaginase activity and asparagine depletion, possibly explaining the less pronounced effect of crisantaspase on coagulation. These effects have a clear impact on dosing requirements, and current data derived from several publications indicate that crisantaspase should be used at a dose of at least 20,000 U/m² on alternate days (or three times weekly) by either the i.v. or i.m. route (22). Crisantaspase was approved by the FDA in November 2011 at dosages of 25,000 U/m² three weekly for six doses by i.m. route. In the expanded access program Erwinaze Master Treatment Protocol (EMTP), 1% of thromboses and <1% bleeding events were recorded in 572 patients (median age 9 years, range 1–66) (http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125359lbl.pdf). Notably, a similar dosage (20,000 U/m² s.c. every other day for six doses) was reported to have irrelevant haemostatic toxicity in 10 adult patients, who had no significantly different changes in fibrinogen, AT, or D-dimer (111). Crisantaspase was found to induce a decrease in coagulation proteins similar to that caused by colaspase in one study comparing 27 patients treated with crisantaspase to 15 patients treated with colaspase Medac 1,000 U/m²; however, in this study, the dose of crisantaspase was as high as 30,000 U/m² for 10 days, i.e. with a frequency and a total dosage double that found without haemostatic toxicity (79).

Prevalence of haemorrhagic and thrombotic complications during asparaginase treatment

Haemorrhagic events

Decreases in fibrinogen and procoagulant factors can enhance the bleeding risk in ALL patients treated with asparaginase. Several cases of patients with intracranial haemorrhages due to severe asparaginase-related hypofibrinogenaemia have been reported (112–114); in a series of 1,547 children with ALL, nine had intracranial bleeding (0.58%) (115). In a meta-analysis of prospective studies, overall bleeding complications occurred in 2% of cases (116). In contrast, bleeding occurred in 31 of 214 adult patients (14.5%), with only one case in the central nervous system (CNS) (in one patient who also had cerebral vein thrombosis [CVT]) (0.46%) (117). No haemorrhages in the CNS were recorded in a series of 238 adult patients (118) or in one series of 719 paediatric patients, one-third of whom received prophylactic fresh-frozen plasma or cryoprecipitate (119).

Thrombotic events

The major part of the occlusive events occurring during asparaginase treatment involve venous vessels. In one retrospective study of 238 adult ALL patients, the rate of asparaginase-related ischaemic stroke was estimated at 0.8% (118); in another series of 214 adult patients, no arterial thrombosis occurred (117). Ischaemic stroke is rare also among paediatric ALL patients, and it occurs in approximately one per 1,000 cases treated with asparaginase (115, 120).

In a prospective study of 60 children, the outcome was either symptomatic or asymptomatic thrombosis. During the follow-up, all of the patients were screened with bilateral venography or magnetic resonance imaging (MRI) of the upper body, ultra- sound of the upper body, echocardiography, and MRI of the head. Twenty-two children had confirmed thromboses, for a prevalence of 36.7%. The locations of the thromboses were the cerebral veins in one patient, the right atrium in three patients, and the upper central venous system in 19 patients. Three patients (5%) presented with clinically symptomatic thromboses (121).

Three narrative reviews and two meta-analyses estimated the rate of symptomatic thrombosis in paediatric and adult patients (116, 122–125). A first review of symptomatic cases, conducted after the exclusion of case reports and the inclusion only of case series or clinical trials, estimated a rate of thrombosis of 4.7% after 1990 (122). A more recent meta-analysis conducted in paediatric
patients estimated in 5,501 patients reported in retrospective studies a rate of thrombosis of 1.5%, and in 1,752 patients reported in prospective studies a rate of thrombosis of 5.2%. In prospective studies, the thrombotic rate was independent of the total dose of asparaginase received and was more frequently associated with lower doses of asparaginase (6,000 U/m² or less, 8%) and/or a length of therapy >9 days (9.6%). Other risk factors for thrombosis were the use of prednisone in the postinduction phase (instead of dexamethasone) and the use of anthracyclines. In addition, the use of the Medac brand of colaspase was found to be associated with a higher rate of thrombosis (8.7%) (116).

In two further large trials in paediatric patients not included in such meta-analyses, the rates of VTE were 3.2% (1.1% CVT) in 1,824 patients (126) and 2.4% (1.6% CVT) in 2,042 patients (120). The patients received in the former trial 4 to 12 doses of pegaspargase 1,000 U/m² and, in the latter trial, eight doses of colaspase 5,000 U/m² (120, 126). In an analysis of the patients treated at the Dana-Farber Cancer Institute between 1991 and 2008, the rate of VTE during treatment phases including asparaginase was 5.4% in paediatric patients and 34% in adult patients, with age as the only predictor of VTE in a multivariate model, including sex, race, leucocyte count and the presence of an anterior mediastinal mass (127). Notably, patients aged 11–16 years old have been reported to have during treatment with colaspase 6,000 U/m² for four doses more severe decreases in procoagulant, anticoagulant, and fibrinolytic factors compared to younger children, so age also seems to predict haemostatic alterations (81).

In a meta-analysis performed in 323 adult patients followed prospectively, the incidence of VTE was 5.9% (123). The risk factors for VTE found in the meta-analysis conducted in paediatric patients (116) seem to be confirmed in the analysis of the adult patients, although the analysis was not sufficiently powered to achieve statistically significant results (123).

In two other studies in adult patients published after the aforementioned meta-analyses, the rates of VTE were 9.3% in 214 patients (CAPELAL study) (117) and 10% in 240 patients (HOVON-37 study) (128); the patients received colaspase 7,500 U/m² on days 10–25 or 5,000 U/m² on days 15–28 of the induction phase, respectively. In the former study, recent use of oral contra-

### Table 3: Incidence of venous thromboembolism (VTE) in paediatric or adult patients with acute lymphoblastic leukaemia (ALL) and treated with colaspase

<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>Studies included</th>
<th>ALL patients</th>
<th>Patients with VTE</th>
<th>CVT</th>
<th>DVT</th>
<th>PE</th>
<th>uDVT</th>
<th>Other sites</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caruso et al., 2006 [116]</td>
<td>Meta-analysis of prospective studies</td>
<td>17</td>
<td>1,752 children</td>
<td>73 (4.2%)</td>
<td>26 (1.5%)</td>
<td>7 (0.4%)</td>
<td>1 (0.05%)</td>
<td>25 (1.4%)</td>
<td>3</td>
<td>The site of 11 VTE events was not specified</td>
</tr>
<tr>
<td>Meister et al., 2008 [129]</td>
<td>Prospective</td>
<td>1</td>
<td>112 children</td>
<td>9 (8%)</td>
<td>3 (2.7%)</td>
<td>3 (2.7%)</td>
<td>0</td>
<td>3 (2.7%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Qureshi et al., 2010 [126]</td>
<td>Prospective</td>
<td>1</td>
<td>1,824 children</td>
<td>59 (3.2%)</td>
<td>21 (1.1%)</td>
<td>4 (0.2%)</td>
<td>0</td>
<td>29 (1.6%)</td>
<td>2</td>
<td>The site of 3 VTE events was not specified</td>
</tr>
<tr>
<td>Grace et al., 2011 [127]</td>
<td>Prospective</td>
<td>4</td>
<td>501 children</td>
<td>27 (5.4%)</td>
<td>7 (1.4%)</td>
<td>5 (1%)</td>
<td>3 (0.6%)</td>
<td>10 (2%)</td>
<td>4</td>
<td>In 2 patients VTE occurred in two sites</td>
</tr>
<tr>
<td>Santoro et al., 2013 [120]</td>
<td>Prospective</td>
<td>1</td>
<td>2,042 children</td>
<td>48 (2.4%)</td>
<td>33 (1.6%)</td>
<td>5 (0.2%)</td>
<td>0</td>
<td>10 (0.5%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ranta et al., 2013 [130]</td>
<td>Retrospective</td>
<td>1</td>
<td>85 children</td>
<td>4 (4.7%)</td>
<td>2 (2.3%)</td>
<td>0</td>
<td>0</td>
<td>2 (2.3%)</td>
<td>1</td>
<td>In 1 patient VTE was recurrent</td>
</tr>
<tr>
<td>Caruso et al., 2006 [123]</td>
<td>Meta-analysis of prospective studies</td>
<td>13</td>
<td>323 adults</td>
<td>19 (5.9%)</td>
<td>n.s.</td>
<td>7 (2.2%)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Hunault- Berger et al., 2008 [117]</td>
<td>Retrospective</td>
<td>1</td>
<td>214 adults</td>
<td>20 (9.3%)</td>
<td>5 (2.3%)</td>
<td>7 (3.2%)</td>
<td>3 (1.4%)</td>
<td>5 (2.3%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grace et al., 2011 [127]</td>
<td>Prospective</td>
<td>1</td>
<td>47 adults</td>
<td>16 (34%)</td>
<td>2 (4.2%)</td>
<td>4 (8.5%)</td>
<td>4 (8.5%)</td>
<td>7 (14.9%)</td>
<td>1</td>
<td>In 2 patients VTE occurred in two sites</td>
</tr>
<tr>
<td>Lauw et al., 2013 [128]</td>
<td>Retrospective</td>
<td>1</td>
<td>240 adults</td>
<td>24 (10%)</td>
<td>9 (3.7%)</td>
<td>3 (1.2%)</td>
<td>1 (0.4%)</td>
<td>11 (4.6%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

CVT: cerebral vein thrombosis; DVT: deep-vein thrombosis of the legs; PE: pulmonary embolism; uDVT: upper extremity deep-vein thrombosis; n.s.: not specified.
Venous thromboembolism
Sites of thrombosis

In the first review of symptomatic cases published after 1990, the majority of thrombotic events (75%) involved venous vessels, of which 28% were in cerebral veins and 43% were thromboses of deep veins. Pulmonary embolism and thrombus in the right atrium occurred in 2% each (122). A meta-analysis of 91 thrombotic events in paediatric patients found a 28.6% rate of CVT, a 7.7% rate of deep-vein thrombosis (DVT) of the legs, a 27.5% rate of DVT of the upper extremities, a 1.1% rate of pulmonary embolism (PE), a 1.1% rate of thrombosis of the right atrium, and a 2.2% rate of superficial vein thrombosis. In the remaining one-third of cases, thrombosis was not clearly specified, but in 25% of cases it involved the CNS (116). In the UKALL 2003 trial of 61 VTE events in paediatric patients, 36% of the events were CVT, and 50% were upper extremity DVT. In the HOVON-37 study of 24 VTE events, 37% were CVT (117, 120, 126–130). In the AIEOP-BFM ALL 2000 trial of 48 VTE events in paediatric patients, 69% of cases were CVT, 21% were upper extremity DVT (19% CVC-related), and 10% were DVT of the legs (n=4), portal vein thrombosis (n=1), and renal vein thrombosis (n=1) (126). In the series from the Dana-Farber Cancer Institute of 47 events (29 in paediatric patients and 18 in adult patients), 19% of cases were CVT, 36% were upper extremity DVT (19% CVC-related), 19% were DVT of the legs, 15% were PE, 1% were intracardiac thrombosis, and 8.5% involved other sites. No statistically significant differences were found in the distributions of the VTE sites between paediatric and adult patients; however, in paediatric patients, the frequency of CVT was twice that of adult patients (24% vs 11%) (127).

Regarding adult patients, in the CAPELAL study of 21 VTE events, 24% of cases were CVT, 24% were upper extremity DVT CVC-related, 38% were DVT of the legs, ad 13% were PE (117), and in the HOVON-37 study of 24 VTE events, 37% of cases were CVT, 46% were upper extremity DVT (128). In conclusion, summarising the more recent data published in detail between 2008 and 2013 and not included in the meta-analyses by Caruso et al. (116, 123), the most frequent VTE events that were asparaginase-related were CVT and DVT of the upper extremities, which were CVC-related (117, 120, 126–130) (Figure 2). In fact, approximately one-third of VTE events were DVT of upper extremities, either in paediatric or adult patients, whereas the most commonly represented manifestation in paediatric patients was CVT (45% of the cases), and in adult patients it was DVT of the legs and pulmonary embolism (36% of the cases) (Figure 2). However, when asymptomatic VTE was analysed, the near uniformity of the events was related to the presence of a CVC (121).

Recurrent venous thromboembolism

Thirty-eight paediatric patients who developed VTE during treatment with pegaspargase 1,000 U/m² (10 of them with CVT) were re-exposed to asparaginase, with two-thirds of the cases receiving prophylaxis with low-molecular-weight heparin (LMWH). No recurrent thromboses or excess bleeding due to heparin was reported (126).

In the patient series from the Dana-Farber Cancer Institute, one-third of the 33 patients restarted on asparaginase after VTE experienced a recurrence (17% of paediatric patients vs 47% of adults, p=0.07) despite concomitant antithrombotic prophylaxis (127).

Fatal venous thromboembolism

Fatalities due to asparaginase-related VTE have been reported rarely. In the great majority of patient series, all of the individuals have survived thrombosis.

In a prospective trial including 301 children with ALL, the rate of VTE was 11%, with half of the cases involving cerebral vessels. One of the 15 patients with CVT died due to secondary intracranial bleeding, with an overall rate of vascular death of 3.3 per 1,000 patients and a rate of fatality of 7% (131). Consistent data were
found in a recent large trial of paediatric patients, with an overall mortality rate due to VTE of 1.5 per 1,000 patients and a rate of mortality among the patients with CVT as high as 9% (120).

In a retrospective survey of adult patients, the rate of VTE was 3.4%, but the mortality rate was reported at 50% with VTE events, due in three cases to CVT and in one case to PE (118). However, this elevated fatality rate might reflect a bias in the reporting of major events. In contrast, in another series of adult patients, the rate of VTE was 9.3%, but no vascular deaths were recorded (117). However, the occurrence of thrombosis is associated with a reduction in the rate of complete remission (128) and both overall and disease-free survival, independent of known prognostic factors for ALL (117).

Role of inherited thrombophilia

Inherited thrombophilia can be caused by two main mechanisms: the loss of function of endogenous anticoagulants (including deficiency or dysfunction of AT, PC or PS); and the gain of function of procoagulant factors. The latter includes mainly factor V Leiden (FVL), which is resistant to the anticoagulant action of activated PC, prothrombin G20210A (PT20210A), which is often associated with high plasma levels of prothrombin, and high levels of factor VIII (96). There is a risk gradient that is higher in AT, PC, and PS deficiencies, homozygous FVL or PT20210A and multiple abnormalities than in heterozygotes for FVL or PT20210A. Accordingly, the former abnormalities are commonly identified as severe thrombophilia and the latter as mild thrombophilia (96).

In a multicentre, prospective study of paediatric ALL patients treated with BFM 90/95 (including colaspase Medac 5,000 U/m² at three-day intervals), 11% of the children suffered VTE during induction. The risk was higher in patients with a prothrombotic defect (46.5% vs 2.2%, p<0.0001); the risk was further enhanced by the presence of multiple prothrombotic factors or a CVC (131). In a recent trial of paediatric ALL patients, laboratory investigations for thrombophilia were performed only in patients who experienced VTE, so no reliable estimate of the associated risk was possible; however, the prevalence rates of FVL and PT20210A were 20% and 10.7%, respectively, which were much higher than the expected values of 5% and 2% in the general population (120). In a meta-analysis, the presence of at least one genetic prothrombotic factor was associated with an 8.5-fold increase in the risk of thrombosis in ALL children (116). In contrast, the role of inherited thrombophilia as a predictor of VTE in this setting has been denied by other studies (121, 132). However, the thrombotic risk associated with thrombophilia is heavily influenced by protocol differences: in the German studies, children with ALL and thrombophilia receiving colaspase in combination with prednisone had a 34.5-fold increased risk of VTE (133), whereas no increase in risk occurred in the absence of concomitant steroid medication (132).

Only one study investigated the role of antiphospholipids as a risk factor for VTE in those patients: in a prospective series of 60 children with ALL, 4 of 22 with VTE had antiphospholipid antibodies, which was not different from the rate in patients not having thrombotic events (4 of 48, p=0.26) (121).

Prevention and treatment of venous thromboembolism

Replacement treatment with fresh frozen plasma

The decreases in procoagulant and anticoagulant factors during treatment with colaspase have prompted the use of replacement strategies to maintain levels of haemostatic factors considered safe, to minimise the risks of both bleeding and thrombosis.

In one study the use of fresh frozen plasma (FFP) has been reported to increase fibrinogen levels (134), but in most studies FFP was ineffective in increasing haemostatic factors and in reducing hypercoagulability (117, 119, 128, 135, 136) (Table 4). In one study, no CVT was recorded in children with ALL replaced with FFP and/or cryoprecipitate, compared to a 1.5% prevalence in those without supplementation. However, the difference was not statistically significant (119); moreover, the two policies (supplementation vs no supplementation) were applied in two different haematological centres, and the distribution of CVT in one only institution could have incurred some centre-driven bias (119). Data obtained in adult patients have been contrasting. In one retrospective study, no clinical efficacy in preventing VTE was reported (117); in another retrospective study, the use of FFP was associated with a significant reduction in the rate of VTE (OR 0.29, 95% CI 0.11–0.76), although supplementation had no effects on plasma AT levels (128) (Table 4). Finally, some concerns have derived from evidence for the presence of free asparagine and glutamine in FFP; consequently, repletion of the asparagine pool with the administration of plasma during asparaginase therapy could lead to a certain antagonising effect on asparaginase action (137).

In the aforementioned HOVON-37 study, 82% of the patients received FFP; in these patients, a non-significant reduction in the rate of complete remission was reported, compared to patients who did not receive FFP (88% vs 96%, p=0.12) (128).

Replacement treatment with AT concentrates

Given the imbalance in haemostasis towards thrombosis and the special decreases in AT levels, replacement with AT concentrates has been employed in several studies (69, 104–107, 117, 121, 129, 130, 134, 138–141) (Table 4). An additional rationale for the use of AT concentrates derives from the evidence that AT replacement can slow thrombin generation in plasma, as shown by decreases in markers of hypercoagulability (106). This effect is even more evident when employing doses of AT not determined by the asparaginase-induced decrease but rather administered at fixed doses, achieving normal or supranormal levels of inhibitor (69, 104, 105).

In some retrospective studies, replacement with AT concentrates to maintain inhibitor levels >50–70% has been reported to be effective in preventing thrombosis (117, 141); this outcome was not confirmed in other studies (107, 121, 130).

In a prospective study children receiving AT concentrates had no VTE if receiving LMWH prophylaxis but developed VTE in 13% of cases if AT concentrates were given in the absence of LMWH (129); indeed, in this study, the median values of circulating plasma AT during supplementation appeared not to exceed...
70%, which is a borderline level recently demonstrated to be associated with a two-fold increased risk of VTE in the general population (142).

All in all, pooling the available data reported in Table 4, a rough estimate of the frequency of VTE in patients receiving AT concentrates is similar to that calculated for patients who did not (6%, 22/364 vs 7%, 60/863). The great heterogeneity of the studies, regarding the AT target level and the schedule of administration, as well as the lack of data obtained from randomised studies, does not support at the present time the use of AT supplementation as a prophylactic measure during asparaginase treatment; it should instead be reserved for the framework of properly designed, randomised investigations.

Table 4: Studies aiming to investigate the haemostatic and/or clinical effects of replacement strategies in patients with acute lymphoblastic leukaemia treated with colaspase.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>ALL patients</th>
<th>Type of replacement</th>
<th>Target</th>
<th>Dose (range)</th>
<th>Replaced patients</th>
<th>Pro-coagulant factors (Fbg)</th>
<th>Anti-coagulant factors (AT)</th>
<th>Markers of coagulability</th>
<th>Thrombosis (replaced vs not replaced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaunschirm et al. 1986 [134]</td>
<td>Prospective</td>
<td>13 children</td>
<td>FFP + AT concentrate</td>
<td>Fbg &gt;1 g/l AT &gt;80%</td>
<td>Mean 52 ml/kg (15–150) 244 U/kg (20–450)</td>
<td>13 (100%) increased</td>
<td>increased</td>
<td>n. a.</td>
<td>0/13</td>
<td></td>
</tr>
<tr>
<td>Halton et al. 1994 [135]</td>
<td>Prospective</td>
<td>8 children</td>
<td>FFP</td>
<td>n. a.</td>
<td>20 ml/kg</td>
<td>8 (100%)</td>
<td>unchanged</td>
<td>unchanged</td>
<td>unchanged</td>
<td>0/8</td>
</tr>
<tr>
<td>Nowak-Göttli et al. 1995 [136]</td>
<td>Prospective</td>
<td>42 children</td>
<td>FFP</td>
<td>Fbg &gt;60 mg/dl</td>
<td>Median 10 ml/kg (5–20)</td>
<td>20 (48%)</td>
<td>unchanged</td>
<td>unchanged</td>
<td>n. a.</td>
<td>n. a.</td>
</tr>
<tr>
<td>Abbott et al. 2009 [119]</td>
<td>Retrospective</td>
<td>719 children</td>
<td>FFP</td>
<td>Cryoprecipitate</td>
<td>AT &gt;50% Fbg &gt;1 g/l</td>
<td>n. a.</td>
<td>FFP 86 (12%) Cmyop. 163 (23%)</td>
<td>n. a.</td>
<td>n. a.</td>
<td>0/86 vs 7/470 (1.5%) 0/163 vs 7/470 (1.5%)</td>
</tr>
<tr>
<td>Lauw et al. 2013 [128]</td>
<td>Retrospective</td>
<td>240 adults</td>
<td>FFP</td>
<td>n. a.</td>
<td>10–15 ml/kg</td>
<td>193 (82%)</td>
<td>unchanged</td>
<td>unchanged</td>
<td>n. a.</td>
<td>12/193 (6%) vs 8/43 (19%) (OR 0.29 95% CI 0.11–0.76)</td>
</tr>
<tr>
<td>Gugliotta et al. 1990 [104]</td>
<td>Prospective</td>
<td>15 adults</td>
<td>AT concentrate</td>
<td>n. a.</td>
<td>2,000 U on alternate days</td>
<td>7 (47%)</td>
<td>increased</td>
<td>increased</td>
<td>normalised</td>
<td>0/7 vs 0/8</td>
</tr>
<tr>
<td>Mattioli Belmonte et al. 1991 [138]</td>
<td>Prospective</td>
<td>30 adults</td>
<td>AT concentrate</td>
<td>not prefixed (n=7) or &gt;40% (n=10)</td>
<td>2,000 U on alternate days or 20–25/kg/day</td>
<td>17 (57%)</td>
<td>unchanged</td>
<td>increased</td>
<td>n. a.</td>
<td>0/17 vs 0/13</td>
</tr>
<tr>
<td>Mazzucconi et al. 1994 [69]</td>
<td>Prospective</td>
<td>25 adults</td>
<td>AT concentrate</td>
<td>not prefixed</td>
<td>50 U/kg/day</td>
<td>25 (100%)</td>
<td>increased</td>
<td>increased</td>
<td>normalised</td>
<td>0/25</td>
</tr>
<tr>
<td>Pogliani et al. 1995 [105]</td>
<td>Prospective</td>
<td>20 adults</td>
<td>AT concentrate</td>
<td>n. a.</td>
<td>1,500 U on alternate days</td>
<td>8 (40%)</td>
<td>n. a.</td>
<td>increased</td>
<td>normalised</td>
<td>0/8 vs 3/12 (25%)</td>
</tr>
<tr>
<td>Nowak-Göttli et al. 1996 [106]</td>
<td>Prospective</td>
<td>27 children</td>
<td>AT concentrate</td>
<td>AT &gt;60%</td>
<td>Median 30 U/kg (20–60)</td>
<td>15 (55%)</td>
<td>n. a.</td>
<td>increased</td>
<td>normalised</td>
<td>0/15 vs 0/12.</td>
</tr>
<tr>
<td>Matsuzaki et al. 2002 [139]</td>
<td>Retrospective</td>
<td>7 children</td>
<td>AT concentrate</td>
<td>AT &gt;70%</td>
<td>Mean 34 U/kg (25–45)</td>
<td>7 (100%)</td>
<td>n. a.</td>
<td>increased</td>
<td>n. a.</td>
<td>0/7</td>
</tr>
</tbody>
</table>
### Antithrombotic prophylaxis with LMWH

The anticoagulant properties of heparin can be hampered in the presence of decreased levels of AT. In the aforementioned study by Meister et al. (129), body weight-adjusted doses of enoxaparin (range 0.75–1.2 mg/kg) administered together AT concentrates were effective in preventing VTE; no major haemorrhagic complications were reported.

In a retrospective study of 41 children diagnosed with ALL in a single centre in Israel between 1994 and 1999 and having received LMWH (median dose 0.84 mg/kg, range 0.45–1.33) starting at the first dose of asparaginase until one week after the last dose, no thrombotic or bleeding events were documented; one boy had a brain infarct one week after discontinuation of LMWH. In an historical control group of 50 patients who did not receive enoxaparin prophylaxis during asparaginase therapy, the rate of symptomatic VTE was 4% (143).

The same group, in a subsequent retrospective study, included 80 consecutive children with ALL newly diagnosed between 1999 and 2008; the administration of enoxaparin (1 mg/kg starting with the first dose of asparaginase until one week after the last dose) was driven by the presence of inherited thrombophilia, which was systematically investigated. Thus, 18 heterozygous carriers of FVL or PT20210A received LMWH prophylaxis; in this group, the rate of VTE was 16.6% (3 of 18, all carriers of PT20210), while the rate was 4.8% (3 of 62) in the patients without thrombophilia not receiving LMWH (144). There was no statistically significant difference in the rate of VTE between the two groups, likely due to the small number of events, but the rate of VTE occurring in thrombophilic children seemed to compare favourably with the 46.5%...
rate of VTE that was reported in thrombophilic children receiving the same protocol (asparaginase and prednisone) in the absence of LMWH prophylaxis (131). No major bleeding was recorded. Finally, in a multicentre study aimed to develop a predictive model for identifying an increased risk of VTE, children without enoxaparin prophylaxis showed significantly reduced thrombosis-free survival, compared to those receiving LMWH (p=0.02) (145). However, such preliminary evidence calls for randomised trials aiming to investigate whether LMWH is beneficial in preventing VTE in this setting.

**Treatment of acute venous thromboembolism**

Evidence-based recommendations for treatment of acute VTE events in patients with ALL are lacking. In several patient series, VTE has been treated with unfractionated heparin or LMWH (117, 120, 126, 127), sometimes followed by vitamin K antagonists (target international normalised ratio [INR] 2 to 3 or modified 1 to 2) (127). The use of AT concentrates accompanying heparin treatment has also been suggested (target plasma level >60%) (124, 127).

Recommendations in this setting have been published by experts and by working groups (124, 125, 146). VTE should be managed over a one- to three-month period with LMWH (twice daily to maintain a 4-hour post-dose anti-Xa level of 0.5–0.8 IU/ml), followed by prophylactic doses (once daily to maintain anti-Xa level of 0.1–0.3 IU/ml) during asparaginase therapy. In cases of extensive CVC-related VTE, the central line can be removed and can be reinserted in a different vein, if necessary (124). In cases of CVT, oral anticoagulants should be considered for 3–6 months (124). However, in current clinical practice in many adult patients, anticoagulation continues until chemotherapy is entirely completed (127).

Thrombocytopenia can occur secondary to the disease or to chemotherapy. Data regarding the platelet count that can be considered safe for full anticoagulation have come from single case reports or small patient series. The following schedule has been proposed: a full dose of LMWH (1 mg/kg twice daily) for the first two weeks, maintaining the platelet count at greater than 50 × 10⁹/l. Subsequently, it is recommended to halve the dose if the platelet count decreases to less than 50 × 10⁹/l or to discontinue it if the platelet count is less than 20 × 10⁹/l (124, 146, 147).

In one series, 2 of 18 adult patients (11%) experienced major bleeding during LMWH: one intracranial haemorrhage and one compartment syndrome after bone marrow aspiration. The dosage of LMWH and the platelet count at the time of bleeding were not reported (127). No major bleeding due to antithrombotic treatment was recorded in another series of 53 children with VTE (126) or in a small series of seven children with CVT (120), neither during acute therapy nor during secondary prophylaxis.

**Future directions**

The *in vivo* efficacy of heparin is in part dependent on AT levels, so it can be impaired during the period of asparaginase-induced AT depression. Failure of primary or secondary antithrombotic LMWH prophylaxis during asparaginase treatment has been reported in up to 16.6% (144) and 33% (127) of patients, respectively. In contrast, no VTE was recorded among patients receiving contemporary LMWH and AT concentrates (129).

Direct thrombin inhibitors (gatrans) or factor Xa inhibitors (xabans) do not rely on AT for the inhibition of coagulation, and they are now largely proven effective in the treatment of VTE (148). One of these direct thrombin inhibitors, melagatran, produced *in vitro* a significant reduction in endogenous thrombin generation in the plasma of children with ALL obtained during induction. In contrast, the anticoagulant action of LMWH was markedly affected by endogenous AT levels (149). The pro-drug xilamegatran, tested in clinical trials, has been withdrawn from the market because of concerns about potential liver toxicity. However, the results from the present study might be used as a model for other direct thrombin or factor Xa direct inhibitors in situations with reduced AT levels. Recently, edoxaban, a direct factor Xa inhibitor effective in the treatment of VTE in humans, was proven to exert a comparable antithrombotic effect even in mice with low plasma AT, similar to that observed in wild-type mice (150).

**Conclusions**

Asparaginase is the cornerstone of treatment for ALL. Nevertheless, the vast majority of data concerning the pathogenesis and incidence of plasma alterations suffer from limitations due to the wide heterogeneity of the drug sources, dosages, and administration schedules; the different designs of the studies with regard to the timing of blood drawings; and the different types of laboratory applications. Despite a half-century of studies on asparaginase treatment for ALL, some elements are still either completely unexplored (e.g. the effect on fibrinolysis investigated by a global test, such as the clot lysis time) or extremely uncertain (e.g. the effects on factor VIII levels, increases in which could act as a strong risk factor for VTE). The great majority of studies aiming to investigate haemostasis perturbation have inevitably been small, so in many cases, any attempts to correlate laboratory findings with clinical evidence have remained elusive.

Venous thrombosis is a major complication of asparaginase treatment; its incidence is relatively low, but in more than one-third of cases, it can include of life-endangering events such as CVT and PE, with a fatality rate in some studies as high as nearly 10%. Moreover, the occurrence of VTE has a negative impact on the achievement of complete remission and on the life expectancy of patients, likely due to the lack of a subsequent optimal chemotherapeutic treatment for the neoplastic disease. Studies aiming to investigate prevention strategies via replacement of the factors decreased by asparaginase and/or by pharmacological prophylaxis have been inconclusive, as they are statistically underpowered and heterogeneous with respect to their schedules of intervention and in their choices of clinical outcomes. Therefore, large multicentre trials are urgently needed to address such important clinical issues, accompanied by well-designed and feasible laboratory work-ups.
In the near future, -gatran and -xaban direct inhibitors will present exciting opportunities for application in this special patient setting of the knowledge gained in the current clinical practice of VTE management in the general population.

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Conflicts of interest

None declared.

References


