The risk of thrombosis in patients with acute leukemia: occurrence of thrombosis at diagnosis and during treatment

Institute of Hematology, Catholic University, Rome, Italy


Summary. Background: Thromboembolism can occur during acute leukemia, especially acute lymphoid leukemia (ALL) treated with L-asparaginase. Yet, most reports are anecdotical and scarce data are available on the risk of thrombosis in acute myeloid leukemia (AML). Objectives: To evaluate the risk of thrombosis in patients with acute leukemia. Patients and methods: Three-hundred and seventy-nine consecutive adult patients with newly diagnosed acute leukemia were recruited in an observational cohort study conducted from January 1994 to December 2003. Diagnosis was ALL in 69 patients, acute promyelocytic leukemia (APL; FAB subtype M3) in 31, and non-M3 AML in 279. All first or recurrent symptomatic thromboembolic events objectively diagnosed were recorded. Results: Twenty-four patients of the overall 379 (6.3%; 95% CI 4.1%–9.2%) had a first thrombosis, venous in 80% of the cases and arterial in 20%. At diagnosis, thrombosis was a presenting manifestation in 13 cases (3.4% of the whole cohort): 1.4% in ALL, 9.6% in APL, and 3.2% in non-M3 AML patients. Follow-up was carried out on 343 patients without thrombosis at diagnosis and further 11 thrombotic events (3.2%) were recorded. At 6 months from diagnosis, the cumulative incidence of thrombosis was 10.6% in ALL, 8.4% in APL, and 1.7% in non-M3 AML patients. The patients who received L-asparaginase had a 4.9-fold increased risk of thrombosis in comparison with those who did not (95% CI 1.5–16.0). The fatality rate due to thrombosis was 0.8%. Conclusions: In patients with acute leukemia, the risk of thrombosis is not negligible. Thrombosis can be a presenting symptom at diagnosis in a significant portion of cases with APL (9.6%) and non-M3 AML (3.2%); a similar rate of thrombosis can occur during the subsequent course of the disease. The incidence of symptomatic thrombosis at diagnosis is relatively low in ALL patients (1.4%), but is significantly increased by further treatment up to 10.6%. Strategies of antithrombotic prophylaxis should be investigated in this setting.

Keywords: acute leukemia, all-trans-retinoic acid, L-asparaginase, thrombosis.

Introduction

Patients with acute leukemia are typically prone to infectious or hemorrhagic complications, frequently life-threatening. Yet, thrombosis has been repeatedly described as possibly complicating the course of the disease; in particular, treatment of acute lymphoid leukemia (ALL) with L-asparaginase is well known to cause an impairment of anticoagulant and fibrinolytic mechanisms, producing a prothrombotic state leading to overt thrombosis in 2%–10% of the patients [1–5]. A search in Medline using the terms ‘acute leukemia’ and ‘thrombosis’ listed 132 papers published from 1973 to 2004, 55% of them concerning thrombosis complicating ALL (mostly during therapy with L-asparaginase) and 23% reporting thrombosis complicating acute promyelocytic leukemia (APL). The introduction of the differentiating agent all-trans-retinoic acid (ATRA) in the treatment of APL allowed achievement of complete remission in more than 90% of the cases and improved dramatically the coagulopathy typical of this disease [6]. The modifications induced by ATRA in the balance between procoagulant and fibrinolytic properties of the pathological promyelocytes before complete differentiation have been hypothesized to induce a prothrombotic effect [7]. This suggestion seems to be supported by the fact that two-thirds of the thrombotic events described in APL and listed in Medline occurred during a concomitant treatment with ATRA. However, papers dealing with thrombosis and acute leukemia report almost exclusively anecdotical cases or uncontrolled historical series. Only five controlled patient series specifically aimed to evaluate the incidence of thrombosis have been published, all concerning ALL patients receiving L-asparaginase: two retrospective investigations (one on children and one on adult patients) [2,8] and three prospective
studies on pediatric patients [4,9,10]. Recently, a retrospective investigation on a large series of patients focused the association between venous thromboembolism (VTE) and acute leukemia before starting chemotherapy [11]. The present study was designed to evaluate the occurrence of symptomatic thrombotic manifestations objectively diagnosed in a series of consecutive patients with newly diagnosed acute leukemia.

**Patients and methods**

**Patients**

This is a follow-up observational cohort study conducted on 379 consecutive patients with newly diagnosed acute leukemia admitted to our institution from January 1994 to December 2003.

Diagnosis of acute leukemia was essentially based on French–American–British (FAB) guidelines by conventional morphocytochemical criteria and immunophenotyping [12–14]. Moreover, diagnosis of APL was confirmed by karyotypic and/or molecular evidence of the t (15;17) chromosome translocation. Acute lymphoid leukemia was diagnosed in 69 cases: 17 with L1 FAB subtype, 44 with L2, and eight with L3. Acute myeloid leukemia was diagnosed in 310 cases: 18 with M0 subtype, 26 with M1, 69 with M2, 31 with M3 (APL), 43 with M4, 25 with M5, 14 with M6, and three with M7. Three cases were classified as biphenotypic leukemia; 50 patients had AML evolved from a myelodysplastic syndrome (MDS) and were not eligible for FAB classification. Finally, the remaining 28 patients had AML with at least bilineage dysplasia at the onset not fitting all the FAB criteria. Acute promyelocytic leukemia had been ruled out in all the cases without FAB classification. The main clinical characteristics of the patients are reported in Table 1.

Three-hundred and twenty-one patients (87%) were treated according to protocols of the Italian co-operative group Gruppo Italiano Malattie Ematologiche dell’Adulto (GIMEMA) adopted at the time of diagnosis [15–24]. The eight patients with ALL of L3 subtype were treated according to Magrath et al. [25]. Nine patients (seven with ALL and two with APL) were aged between 14 and 18 years and were included in this cohort according to the eligibility criteria established for the GIMEMA ALL protocols (age ≥ 14 years) and for the GIMEMA AIDA protocols (age ≥ 1 year) for treatment of APL.

All protocols included induction and consolidation intensive treatments based on the administration of various chemotherapeutic agents by different schedules (essentially anthracyclines, cytosine arabinoside, and etoposide in all trials; the ALL patients received besides the drugs above mentioned also vincristine, prednisone, cyclophosphamide, and methotrexate). The majority of patients with non-L3 ALL (72%) underwent treatment regimens which included in the induction phase schedules with t-asparaginase 6000 U m⁻² administered within the first 30–45 days from diagnosis for a total of 7–10 doses [19–21]. All the patients with APL were treated according to the GIMEMA AIDA protocols and received a regimen including ATRA ranging from 25 (in patients younger than 20 years or older than 60 years) to 45 mg m⁻² [22,23]. The remaining patients not eligible for inclusion in the GIMEMA trials because aged more than 60 years (when constituting an exclusion criterion) or for the presence of major comorbidity were treated with non-aggressive approaches or palliative chemotherapy or supportive care [26,27].

Patients started treatment as soon as diagnosed and underwent a strict clinical and laboratory follow-up according to established procedures for management of acute leukemia. They received standard antibiotic and supportive care; prophylactic platelet transfusions were given according to a threshold of a platelet count less than 10.0 × 10⁹ L⁻¹ (or 20.0 × 10⁹ L⁻¹ in the case of fever or bleeding) [28]. Oral tranexamic acid 1 g every 6 h was given until the platelet count was less than 20.0 × 10⁹ L⁻¹. No patient was given primary antithrombotic prophylaxis.

**Study end points**

Since 1994, all clinical and laboratory data concerning the patients with acute leukemia admitted to our department have been registered on a database having a devoted section for the occurrence of thrombotic events objectively diagnosed.

The diagnosis of venous thrombosis of extremities was adjudicated only when based on objective methods such as compression ultrasonography or color Doppler ultrasonography; diagnosis of superficial vein thrombosis (SVT) also was objectively established by ultrasonographic examination. Diagnosis of pulmonary embolism (PE) was considered valid on the basis of perfusion lung scanning or computed tomography (CT). Occlusion of cerebral or abdominal vessels was diagnosed with magnetic resonance imaging or CT. All the causes of death attributed to thrombosis were confirmed by necropsy. SVT of the arms, possibly due to diagnostic or therapeutic phlebotomy, were not computed as events of interest.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients</th>
<th>Sex: M/F</th>
<th>Age, years: median (range)</th>
<th>Observation time, months: median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>69</td>
<td>29/40</td>
<td>47 (14–80)</td>
<td>11 (1–86)</td>
</tr>
<tr>
<td>AML M3</td>
<td>31</td>
<td>15/16</td>
<td>51 (16–75)</td>
<td>25 (1–78)</td>
</tr>
<tr>
<td>Non-M3 AML</td>
<td>279</td>
<td>156/123</td>
<td>63 (18–89)</td>
<td>7 (1–74)</td>
</tr>
<tr>
<td>ALL + AML</td>
<td>379</td>
<td>200/179</td>
<td>60 (14–89)</td>
<td>7 (1–86)</td>
</tr>
</tbody>
</table>

© 2005 International Society on Thrombosis and Haemostasis
Symptomatic thrombotic events were systematically recorded soon after their validation by the responsible physicians (see list of contributors); treatment was essentially based on the administration of s.c. low molecular weight heparin (LMWH) (enoxaparin) 100 U kg\(^{-1}\) b.i.d.; in the case of platelet count lower than 50.0 \(\times 10^9\) L\(^{-1}\) or in the clinical suspicion of bleeding risk treatment was reduced to 100 U kg\(^{-1}\) once a day or 50 U kg\(^{-1}\) b.i.d.; alternatively, i.v. unfractionated heparin was given at dosages aimed to obtain an aPTT value in the lower therapeutic range (1.5 \(\times\) normal value). Secondary prophylaxis after deep vein thrombosis was essentially based on the administration of LMWH 100 U kg\(^{-1}\) once a day in the case of ongoing chemotherapy or standard oral anticoagulation (INR 2.0–3.0) otherwise. In general, the length of secondary prophylaxis did not exceed 6 months.

All patients with thrombosis underwent laboratory screening for inherited thrombophilia as previously described [29,30]. Laboratory evaluation included measurement of antithrombin and protein C activities, free protein S antigen levels, and fasting homocysteine. DNA samples were investigated for the presence of factor (F)V Leiden or the G20210A substitution in the prothrombin gene. Systematic laboratory investigation was carried out only in the patients who suffered from thrombosis.

**Statistical methods**

Statistical analysis was performed by the GB-STAT software system V6.5 (Dynamic Microsystems, Silver Spring, MD, USA).

The incidence of thrombosis at the onset of disease and before starting treatment was estimated on the whole cohort of patients. Differences between groups were estimated by the Fisher’s exact test or the chi-squared test or the Mann–Whitney U-test used when appropriate (statistical significance for \(P < 0.05\)). Patients were stratified according to diagnosis of ALL, or APL, or non-M3 AML, sex, age \(> 45\) years, leukocyte count \(> 50.0 \times 10^9\) L\(^{-1}\), platelet count \(> 30.0 \times 10^9\) L\(^{-1}\).

After exclusion of the patients who had thrombosis as one of the presenting manifestations before starting chemotherapy and/or exclusion of the patients who died early (within 2 weeks from diagnosis), the cumulative incidence of first thrombosis was estimated according to the Kaplan–Meier method on the remaining patients who had an observation time of at least 1 month.

The observation time was defined as the time between the diagnosis of acute leukemia with admission to our hospital department and the thrombotic event of interest (uncensored observations); alternatively, the end of the observation time was defined by the date of the death, the date of the last control for the patients lost to follow-up, or the date of the end of the study (censored observations). A Cox proportional hazards regression model was calculated with thrombosis as the dependent variable in function of the time after stratification of the patients for diagnosis (ALL, or APL, or non-M3 AML), sex, age \(> 45\) years, administration of intensive chemotherapy or palliative therapy, administration of l-asparaginase, and administration of ATRA.

To compare thrombosis-free survival curves, the Wilcoxon test, which places more weight on early events, was used. The confidence intervals of the hazard ratio values were calculated according to Simon [31].

**Results**

Fifty-nine patients out of 379 (15.6%) were alive at the end of the study; 25 patients (6.6%) died early (within 2 weeks from diagnosis), 105 patients (27.7%) died within 1–6 months from diagnosis of acute leukemia, and other 165 patients (43.5%) afterward. Twenty-five patients (6.6%) were lost to follow-up, but none of them before 6 months from the diagnosis. Accordingly, 354 patients (93.4%) had a minimum observation time of 1 month and 219 (57.8%) completed a follow-up of 6 months.

**Thrombotic events**

Twenty-four patients of the total cohort (6.3%; 95% CI 4.1%–9.2%) had a first symptomatic thrombosis, in 13 cases (54.1%) occurring at the time of diagnosis of acute leukemia and before starting any cytoreductive treatment. No patient had suffered from venous or arterial thrombosis before diagnosis of acute leukemia. The main characteristics of the patients with thrombosis are summarized in Table 2: diagnosis was deep venous thrombosis (DVT) of the arm in one case, DVT of the leg in 14 (in four cases associated with PE), SVT of the leg in two, cerebral venous thrombosis in one, portal vein thrombosis in one, and arterial ischemic arterial stroke in five (fatal in two cases). First DVT of the leg was distal in two cases, one of them associated with PE, and proximal in the remaining 12. Overall, only three out of the 24 recorded first thromboses could be labeled as minor events (one distal DVT of the leg without embolization and 2 SVTs). Five patients had a recurrent thrombosis (four DVTs of the legs and one fatal PE) during remission of the disease (Table 2). Three patients (0.8%) died for thromboembolism: two, aged 69 and 84, respectively, for arterial ischemic stroke at diagnosis, and one, aged 51, for recurrent PE. Fatality for thrombosis accounted for 1% of all the recorded deaths (three out of 295).

**Incidence of thrombosis at the diagnosis**

Thrombosis was a clinical presenting manifestation in 13 patients of 379 (3.4%, 95% CI 1.8%–5.8%): one with ALL (1.4%), three with APL (9.6%), and nine with non-M3 AML (3.2%). After exclusion of the four patients with arterial ischemic stroke (two with APL and two with non-M3 AML), the incidence of VTE at diagnosis was 3.2% in APL and 2.5% in non-M3 AML. In two cases, thrombosis was the cause of early death (8.0% of all the early deaths) (Table 2). No significant difference was found in the incidence of thrombosis among the patient groups stratified according to the type of.
leukemia (ALL or APL or non-M3 AML, \(P = 0.10\)), sex (\(P = 0.15\)), age > 45 years (\(P = 0.33\)), leukocyte count > 50.0 \(\times 10^9\) L\(^{-1}\) (\(P = 1.0\)), platelet count > 30.0 \(\times 10^9\) L\(^{-1}\) (\(P = 1.0\)).

The patients with thrombosis as presenting manifestation had a median leukocyte count at diagnosis of 25.6 \(\times 10^9\) L\(^{-1}\) (range 1.9–92.6), with no statistically significant difference in comparison with the patients without thrombosis (median 11.0, range 0.3–400.0, \(P = 0.38\)). The median platelet count at diagnosis among patients with thrombosis (33.0 \(\times 10^9\) L\(^{-1}\), range 5.0–244.0) was similar to that of the patients without thrombosis (44.0 \(\times 10^9\) L\(^{-1}\), range 1.0–406.0, \(P = 0.92\)).

**Incidence of thrombosis during the course of the disease**

After exclusion of the 13 patients with thrombosis as presenting manifestation and the 23 patients who died early for other causes than thrombosis, follow-up was carried out on the remaining 343 patients (65 with ALL, 252 with non-M3 AML, and 26 with APL) for a total observation time of 439.3 years. Eleven thrombotic events were recorded in the patient cohort (3.2%, 95% CI 1.6%–5.6%), all but one involving the venous vessels (Table 2). All events occurred within 6 months from diagnosis. After the stratification of the patients according to the type of leukemia, the cumulative incidence of thrombosis at 6 months was 10.6% in ALL, 8.4% in APL, and 1.7% in non-M3 AML. The patients with ALL had a higher probability of thrombosis in comparison with the patients with non-M3 AML (hazard ratio 6.8, 95% CI 1.7–27.2, \(P < 0.001\); similarly, the incidence of thrombosis in the patients with APL was higher than in the patients with non-M3 AML (hazard ratio 5.6, 95% CI 0.9–33.5, \(P = 0.01\)) (Fig. 1). The risk for thrombosis was quite similar in the ALL patients and the APL patients (hazard ratio 1.2, 95% CI 0.2–5.9, \(P = 0.84\)). After the exclusion of the two patients with SVT of the leg and the patient with arterial ischemic stroke, the risk for VTE was still significantly higher among the ALL patients and the APL patients in comparison with the patients with non-M3 AML, yet with considerably higher confidence intervals (hazard ratio 16.9, 95% CI 2.0–143.3, \(P < 0.001\), and 17.1, 95% CI 1.5–189.0, \(P < 0.001\), respectively). No significant difference was found in the cumulative incidence of thrombosis after stratification of the patients according to sex (\(P = 0.40\)), age > 45 years (\(P = 0.93\)), administration of intensive or palliative therapy (\(P = 0.99\)), administration or not of ATRA (\(P = 0.17\)).

![Table 2](image_url)
Thrombosis occurred in five of the 45 patients who received L-asparaginase and in six of the 298 ones who did not, with a cumulative incidence at 6 months from diagnosis of 12.0% and 2.7%, respectively; the risk of thrombosis was significantly higher in the patients who were treated in comparison with those who were not (hazard ratio 4.9, 95% CI 1.5–16.0, P = 0.002) (Fig. 1). However, VTE occurred soon after administration of L-asparaginase during the induction phase only in two ALL patients, one of them carrying FV Leiden (Table 2).

Overall, two of the 24 patients with thrombosis (8.3%) carried heterozygosity for the G2010A substitution in the prothrombin gene and for FV Leiden, respectively. Thus, the incidence of inherited thrombophilia in the patients with VTE resulted 10.5% (2 of 19). A definite circumstantial risk factor was found only in the patient with axillary vein thrombosis, occurred after insertion of a central venous line (Table 2).

The overall incidence of thrombosis (both at the onset of the disease and during treatment) in patients with AML of established M4 and M5 subtypes (6 of 68, 8.8%) was higher than in patients with AML of established M0, M1, and M2 subtypes (4 of 113, 3.5%), yet without reaching statistical significance (P = 0.17).

Discussion

Thromboembolism is a well recognized complication of malignancy. The pathogenesis of the cancer-related prothrombotic state is complex and reflects the action of different mechanisms, including activation of blood coagulation via procoagulant substances, impairment of fibrinolytic pathways, alterations of endothelium toward a thrombogenic state detectable in the large majority of patients with cancer [32,33]. In recent years, in vitro and in vivo studies produced relevant insight into prothrombotic mechanisms occurring in acute leukemia, particularly in APL [6,34–38]. In spite of increasing knowledge in this field, scarce systematic data are available concerning the possible clinical impact of thromboembolic disease on the course of leukemic disease. The improvements in therapeutic agents and in the supportive care over the past decades produced a significant progress in treating patients with acute leukemia, but the introduction of novel therapeutic agents accompanied by a decrease in early deaths could disclose novel clinical issues. This has been the case for ATRA, which produces a downregulation of the procoagulant substances expressed by APL cells [34,35], with a rapid amelioration of the coagulopathy [34,36–38] and a dramatic decrease in the rate of the early hemorrhagic death typical of this disease [6]. Yet, in some patients, this has been reported to be accompanied by a contemporary emerging prothrombotic effect with a persistent slight increase of the clotting activation markers, maybe due to an upregulation of the production of cytokines [6,7,36–38].

The only controlled studies aimed to investigate the incidence of thrombosis complicating the treatment of patients with acute leukemia have been carried out in the setting of ALL trials. In patients receiving L-asparaginase, the rate of symptomatic venous thrombosis was reported as extremely wide, ranging from 0.8% to 11.1% [2,4,5,8,9,39]; an estimate considerably higher (36.7%) included also asymptomatic events systematically investigated at the end of the L-asparaginase treatment [10].

In our series of consecutive patients, all the symptomatic thrombotic events objectively diagnosed had been systematically recorded over the past decade. We estimated a 6.3% overall incidence of thrombosis, mostly involving the venous vessels (80% of the cases); 3.4% of all the patients suffered from thrombosis as one of the heralding manifestations of the disease, with a higher incidence among patients with APL (9.6%). The inclusion in our study of unselected patients consecutively diagnosed renders our cohort representative of the adult patient population with acute leukemia. The present investigation is susceptible of underestimation, recording only symptomatic thromboses objectively diagnosed, so that a number of minor asymptomatic occlusive events could have been missed; accordingly, the lower confidence limit of our estimate is highly reliable, giving for the whole cohort an incidence of thrombosis associated with leukemia of at least 4%. This confirms the findings recently obtained by a retrospective investigation on a large cohort of 719 patients.
with acute leukemia, in which VTE was the presenting manifestation in 2.1% of the cases, with no difference between AML and ALL, and with an incidence of 6.5% thrombosis among APL patients [11]. Previous reports claimed an incidence of thrombosis in patients receiving ATRA as high as 10% [40] to 19% [41]; on the opposite, in other series, the incidence of thrombosis was considerably lower [22,42]. In our series ATRA treatment was concomitant with vessel occlusion only in two of the five APL patients suffering from thrombosis, with no significant association with the thrombotic risk assessed during the follow-up. Accordingly, a major role in determining thrombosis in APL could be attributed to the hypercoagulability typical of the disease rather than to ATRA.

Our findings do not firmly support the hypothesis of a special role of hyperleukocytosis as triggering factor for vessel occlusion in acute leukemia [43,44], in agreement with other systematic observations [11].

Our data obtained in ALL patients fit with previous reports estimating as 10% the incidence of symptomatic thrombosis among ALL children receiving l-asparaginase and prednisone [4,5,39]. In a retrospective investigation on adult patients recruited in the GIMEMA ALL 0288 trial, the rate of thrombosis after start of l-asparaginase was found lower (4.2%) [2]. However, in this survey, the fatality rate among the recorded cases with vascular complications was as high as 50%, inducing suspicion of a report bias by the participating centers with possible undersizing of the minor thrombotic events and underestimation of the overall incidence of thrombosis. In the present investigation, l-asparaginase treatment is confirmed to be significantly associated with the risk of developing thrombosis, yet a strict temporal relationship was noticed only in two ALL patients.

A major limitation of our study is the lack of a systematic laboratory screening for thrombophilia, which was offered only to patients with symptomatic thrombosis. This rendered us unable to estimate by a case-control comparison the risk associated with thrombophilia in this patient setting. Inherited thrombophilia, namely FV Leiden and prothrombin G20210A, was detected in 10% of our adult patients with venous thrombosis; however, this estimate is obtained on a sample of only 19 subjects, rendering not meaningful any comparison with the incidence rates obtained on larger samples from patients with VTE or control subjects [29].

In conclusion, in our series of consecutive patients, more than half thrombotic events occurred as presenting manifestation before starting any treatment, giving further evidence in the absence of any publication bias that in leukemic patients vascular occlusion can constitute a complication potentially fatal independently of chemotheraphy or supportive measures [45–50]. In particular, treatment seems to constitute a relatively weak additional triggering factor in the APL patients and in AML patients, in comparison with the time of the first clinical presentation. On the opposite, in ALL patients, l-asparaginase treatment is confirmed to play a major role in favoring thrombosis. Further prospective investigations are warranted in order to refine the estimate of the thrombotic risk associated with acute leukemia, to set up the possible role of inherited thrombophilia, and finally to set up possible antithrombotic prophylactic strategies, especially in patients receiving high-risk treatments.

Contributors
V. De Stefano conceived and designed the study and was responsible together with E. Rossi of the statistical analysis, the final interpretation of the data, and the final drafting of the manuscript; F. Sorì and E. Rossi were responsible for the database collecting the laboratory and clinical data; P. Chiusolo was responsible for the genotype analysis; G. Zini was responsible for the diagnosis of acute leukemia; G. Leone, S. Sica, L. Pagano, P. Chiusolo, L. Laurenti, F. Sorì, and L. Fianchi were the physicians responsible for the recruitment, the treatment and the follow-up of the patients, including adjudication of the thrombotic events; G. Leone critically revised the paper and gave important intellectual contribution. All authors were involved in the final revision of the article, giving contribution to the final interpretation of the data and final approval.

Acknowledgement
This study was supported by a grant from the Funds of the Catholic University.

References
27. Falanga A, Iacoviello L, Evangelista V, Belotti D, Consorini R, D’Orazio A, Robba L, Donati MB, Barbui T. Loss of blast cell pro-

© 2005 International Society on Thrombosis and Haemostasis


