Thromboembolic events in children with acute lymphoblastic leukemia (BFM protocols): prednisone versus dexamethasone administration

U. Nowak-Göttl¹, E. Ahlke¹, G. Fleischhack², D. Schwabe³, R. Schobess⁴, C. Schumann¹, R. Junker⁵.

Pediatric Hematology/Oncology, University Hospitals Münster¹, Bonn², Frankfurt³, and Halle⁴, Institute of Clinical Chemistry and Laboratory Medicine and Institute of Arteriosclerosis Research⁵, University Hospital Münster, Germany

Correspondence: Prof. Dr. Ulrike Nowak-Göttl
Ped. Hematology/Oncology
University Children’s Hospital
Albert Schweitzer Str. 33
D- 48149 Münster, Germany
Tel: + 49-251-8347783
Fax: + 49-251-8347828
Email: leagottl@uni-muenster.de
Alterations in hemostasis leading to symptomatic thromboembolism have been observed in patients with acute lymphoblastic leukemia (ALL) receiving E. coli asparaginase (CASP) combined with steroids. Moreover, hereditary prothrombotic risk factors are associated with an increased risk of venous thromboembolism in pediatric ALL patients treated according to the BFM 90/95 protocols (including CASP combined with prednisone during induction therapy).

To assess whether the thromboembolic risk associated with established prothrombotic risk factors is modified by treatment modalities (prednisone or dexamethasone), the present analysis was performed.

336 consecutively recruited leukemic children treated according to different BFM protocols (PRED group, n=280, prednisone 60 mg/m²; DEXA group, n=56, 10 mg/m² during induction therapy) were studied. Study endpoint was the onset of symptomatic vascular accidents during induction therapy.

The cumulative thromboembolism-free survival was significantly reduced in children within the PRED group (thrombosis frequency: 10.4%) compared with children in the DEXA group (1.8%; p=0.028). Although no significant difference was found in the overall prevalence of prothrombotic risk factors, in the PRED group, 46.5% of the patients suffering a thromboembolic event were carriers of a prothrombotic risk factor, whereas in the DEXA group, no carrier suffered a thromboembolism. At the time of maximum CASP activity, fibrinogen concentrations as well as activities of antithrombin, plasminogen, and protein S were significantly reduced in the PRED group. No significant correlation could be found between CASP activity and levels of coagulation factors.

In conclusion, the use of dexamethasone instead of prednisone, administered with CASP, significantly reduces the onset of venous thromboembolism.

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Alterations in hemostasis have been frequently observed in patients with ALL, and thromboembolic accidents are well documented in children receiving E. coli asparaginase (CASP) as a single agent or in combination with vincristine or steroids, sometimes complemented by an anthracycline.\textsuperscript{3-7} Recently published data suggest that the factor II (FII) G20210A and the factor V (FV) G1691A mutations, deficiencies of protein C, protein S, antithrombin, elevated lipoprotein (Lp)(a) and von Willebrand factor (vWF) concentrations are associated with venous thromboembolism in pediatric patients with acute lymphoblastic leukemia (ALL) treated according to the BFM 90/95 protocols. In these protocols, CASP combined with prednisone was used during induction therapy.\textsuperscript{1,7} In addition, we have demonstrated that children with ALL treated with different leukemia protocols carried a similar rate of the aforementioned prothrombotic risk factors but suffered a significantly lower rate of severe thrombotic events: 11.5% symptomatic venous thromboses occurred within the BFM 90/95 protocols versus only 2.0% within the COALL 92/97 protocols.\textsuperscript{2} Thus, the carrier state of inherited prothrombotic risk factors and treatment modalities have an impact on the risk of thromboembolism in children with ALL.

To assess whether the risk of vascular occlusions associated with established prothrombotic risk factors is additionally modified by specific treatment modalities, especially by prednisone or dexamethasone, the present analysis on prospectively enrolled children with ALL treated with the BFM 90/95 protocol (data already published in 1999)\textsuperscript{1} and the new BFM 2000 protocols was performed.

PATIENTS AND METHODS

\textit{Ethics}

The present multicentre study was performed in accordance with the ethical standards laid down in a relevant version of the 1964 Declaration of Helsinki and approved by the medical ethics committee at the Westfälische Wilhelms-University, Münster, Germany.
Patients

With parental consent, children ≥12 months of age with acute onset of ALL treated according to the BFM 90/95 or the BFM 2000 induction protocols between December 1994 and January 2002 were included in this prospective multicentre analysis. Subjects without complete remission of the disease (day 33 of the induction protocol), children classified as high-risk patients, children with concomitant chronic diseases, hepatic failure or severe septicemia, and adolescents with oral contraceptives or nicotine abuse were excluded from the survival analysis. In addition, children receiving heparin prophylactically (individual decisions by the participating centers) were excluded from the thromboembolism-free survival analysis. The participating centers were from northwestern or eastern Germany.

In total, 336 newly diagnosed leukemic children (median/range of age: 5.1/1-18 years; male n=179, female n=157) were prospectively enrolled in this study. 280 of them were treated according to the BFM 90/95 protocols, and 56 according to the BFM 2000 protocol (pilotized n=47; randomized n=9).

Study end points

The diagnosis of symptomatic venous thromboembolism during ALL induction therapy was defined as the end point of this study. Thrombotic events occurring outside induction therapy were recorded on an explorative basis.

Imaging methods used

Venous thromboembolism was diagnosed if echogenic material was found within the lumen of a vein on gray scale and if partial or complete absence of flow was demonstrated by pulse-wave and color Doppler sonography in a symptomatic patient. Alternatively, venography was used in children with suspected vascular occlusion in the upper extremity, and cerebral venous thromboses were diagnosed with magnetic resonance imaging or computed tomography.
Leukemia therapy

Children in the ALL-BFM 90/95 protocols (PRED group) received CASP medac (Kyowa, Hakko, Kyogo, Japan) in doses of 5000 U/m² at 3-day intervals, starting on day 12 through to day 33 (eight doses). Prednisone (60 mg/m²) on days 1-36 and weekly vincristine (1.5 mg/m²) as well as daunorubicine (30mg/m²) on days 8 and 15 (standard risk) and also on days 22 and 29 (medium risk) were additional elements of therapy. Further, children received prophylactically intrathecal methotrexate on days 1, 12, 30, 45 and 59 during induction therapy. In reinduction therapy the children received CASP medac (10,000 U/m²) on days 8, 11, 15 and 18, along with DEXA (10 mg/m²) on days 1-21, weekly vincristine (1.5 mg/m²) and doxorubicin (30 mg/m²) on days 8, 15, 22 and 29 respectively.\(^1\)

In the BFM 2000 protocol (DEXA group) patients received DEXA 10 mg/m² on days 8 to 29 during induction therapy (instead of PRED on days 1-36 in BFM 90/95). The administration of CASP, vincristine and daunorubicine was no different from the BFM 90/95 protocols. In reinduction therapy children with standard risk received CASP 10,000 U/m² on days 8, 11, 15 and 18, along with dexamethasone (10 mg/m²) on days 1-21 and vincristine (1.5 mg/m²) and doxorubicin (30 mg/m²) on days 1 and 8 respectively. Medium-risk patients received this reinduction protocol for a second course 10 weeks later.

Depending on the individual decisions by the participating centers, children’s polychemotherapy was administered via peripheral veins, or via Broviac, Hickman or Porth catheters implanted within the first weeks of therapy.

Laboratory analyses

The FII G20210A, the FV G1691A mutations, and the plasminogen activator inhibitor-1 4G/4G polymorphism, APC-resistance (APC-R), fibrinogen, antithrombin, plasminogen, Lp(a), protein C, and protein S were investigated with standard laboratory techniques at leukemia onset.\(^1,2,9\) VWF was determined by ELISA technique (Asserachrom, Stago, Asnieres-sur Seine, France). Additionally, in patients with thrombosis factor VIIIC
coagulant activity and factor XII were assayed with deficient plasma from Dade-Behring (Marburg, Germany) in samples obtained prior to the thrombotic event. The plasmatic coagulation factors were again investigated in a subgroup of 57 children (PRED n=25; DEXA n=32) at days with maximum CASP activities (between days 27 to 33 during induction therapy). Classification of deficiency states and risk cut-offs were performed as recently described.\textsuperscript{1,2}

Statistics

All statistical analyses were performed using the StatView 5 software package (SAS Institute Inc.). The probability of a symptomatic thromboembolic event as a function of time was determined with the method of Kaplan and Meier. The log rank test was used to compare the thromboembolism-free survival in ALL patients treated with PRED or DEXA during induction therapy. $\chi^2$ and Fischer's exact test were used to compare the rates of prothrombotic risk factors in children treated with PRED and DEXA. Continuous data are presented as medians and ranges and evaluated by non-parametric statistics using the Wilcoxon-Mann-Whitney U test. The correlation between CASP activities and levels of coagulation factors was tested using Spearman rank analysis.

RESULTS

Prothrombotic risk factors

No significant difference was found in the overall prevalence rate of prothrombotic risk factors in the Caucasian populations studied (p=0.72): The heterozygous FII G20210A mutation was found in 2.3% of the PRED group and in 3.5% of the children treated with DEXA (p=0.63); the FV G1691A mutation was present in 7.1% of both groups (p=0.90); inherited protein C deficiency was found in 2.1% of the PRED group versus 1.8% of the DEXA group (p=1.0); inherited protein S deficiency was confirmed in 1.1% of the PRED group and antithrombin deficiency was reported only once in children treated with PRED.
Elevated Lp(a) (>30 mg/dl) was diagnosed in 6.5% of children treated with PRED compared with 7.1% in the DEXA arm (p=1.0).

*Presence of central lines*

In 249 out of 280 children (88.9%) in the PRED group and in 51 out of 56 in the DEXA group (91.0%) polychemotherapy was administered via central lines (p=0.48). Compared with children in the PRED group no symptomatic central-line-associated thromboembolism was observed (PRED n=18: 7.2%), and no thromboembolism-associated death occurred in the DEXA group (PRED n=1: 0.4%). The rate of Broviac, Hickman or Porth catheters implanted was no different between the two groups investigated.

*Thromboembolic events during induction therapy*

Compared with 29 patients with symptomatic vascular accidents in the PRED group (10.4%), only one nine-year-old girl out of 56 children receiving DEXA (1.8%) suffered symptomatic cerebral venous thromboembolism on day 33 of induction therapy. In this patient, however, hypoplasia of the superior sagittal sinus had been diagnosed prior to leukemia onset. Whereas 24 out of 29 thrombosis in the PRED group were associated with at least one prothrombotic risk factor,¹ no inherited prothrombotic risk factor could be identified in the nine-year-old girl suffering thrombosis in the DEXA group: Fibrinogen, antithrombin, protein C, protein S, plasminogen and Lp(a) were within the normal range at the time of symptomatic thromboembolic onset. In addition, in children with thrombosis factor VIIIIC activities were normal prior to symptomatic thrombotic onset.

As recently reported in PRED treated children, 15 of 29 children suffered cerebral venous thrombosis, five times associated with a central line placed in the internal jugular vein, 12 of 29 patients showed catheter-related superior caval vascular occlusion, and thrombosis of femoral and pelvic veins was diagnosed in another two patients respectively.¹ Children with
cerebral sinus thrombosis initially presented with headache, coma, vomiting or seizures. Patients with symptomatic catheter-related thrombosis in the upper venous system showed swelling of the corresponding extremities (n=4) or neck tissues (n=2), and superficial vein collaterals were observed in three children. In the remaining three patients loss of catheter-patency was the leading symptom of vascular obstruction. The two patients with thrombosis of the lower extremity initially presented with blue and swollen legs, with superficial collateral circulation additionally visible in one patient. The nine-year-old girl in the DEXA group presented with headache and seizures at thrombotic onset.

The significantly reduced cumulative thrombosis-free survival in children treated with PRED compared with children in the DEXA group during ALL-induction therapy (p=0.028) is shown in Figure 1.

![Figure 1. Thrombosis-free cumulative survival in children with ALL: PRED versus DEXA during induction therapy.](image-url)
Thromboembolic events not occurring during induction therapy

Of all patients receiving re-induction therapy in the PRED group 1.7% (n=3) developed symptomatic thromboembolism in the central nervous system during reinduction therapy with CASP and dexamethasone administration. In the DEXA group, two (3.6%) thromboembolic events were observed: a 10-year-old boy (medium risk) suffered venous sinus thromboembolism directly after cessation of dexamethasone application during the second reinduction therapy (day 20). At a similar time point, day 27 during the second re-induction course and three days after intrathecal application of 12 mg methotrexate, a further 13-year-old girl (medium risk) suffered methotrexate-related central nervous system-toxicity mimicking a stroke-like episode with symptoms lasting for approximately 10 days. No further patient in the entire study group suffered a stroke-like episode. Interestingly, both children had a positive family history of recurrent venous thromboembolism. Therefore, a comprehensive laboratory diagnostic work-up was performed in these patients. In the 10-year-old boy, elevated Lp(a), homozygous plasminogen activator inhibitor-1 4G/4G polymorphism and factor XII-deficiency were found, and the 13-year-old girl carried the heterozygous FV G1691A mutation.

Plasmatic coagulation factors at maximum CASP activities

Figure 2 shows medians of maximum CASP activities (between days 27 and 33) and individual CASP values (circles) available in five children prior to symptomatic thromboembolic onset (PRED n=4; DEXA n=1). Concentrations of fibrinogen and free protein S, as well as activities of antithrombin, protein C and plasminogen at days of maximum CASP activities are shown in the Table. Significantly lower values of fibrinogen, antithrombin, plasminogen and free protein S antigen were found in the PRED group. VWF was significantly higher in children treated with DEXA. No difference was found for protein C activities. Correlation analysis between CASP activities and levels of coagulation factors
revealed no significant results (fibrinogen $p=0.2$; antithrombin $p=0.4$; protein C $p=0.3$; protein S $p=0.7$; plasminogen $p=0.1$).

Figure 2. Median (range) values of maximum CASP activity (days 27-33) in children during induction therapy: BFM 90/95 protocol (PRED group) versus BFM 2000 protocol (DEXA group) and individual CASP values (circles) available in five children prior to symptomatic thromboembolic onset (PRED $n=4$; DEXA $n=1$).

DISCUSSION

The present prospective multicentre study in children with ALL was performed to evaluate the risk of vascular occlusions in children treated according to BFM protocols and receiving either dexamethasone or prednisone (BFM 2000: dexamethasone $10\text{mg/m}^2$ (DEXA group); BFM 90/95: prednisone $60\text{mg/m}^2$ (PRED group)) during leukemia induction therapy. Besides the detection of inherited thrombophilic risk factors, the aim of this study was to investigate
further coagulation proteins which might possibly be modified by the specific treatment modalities.

The recorded rate of 1.8% thromboembolic events within the DEXA group was within the lower range of published data in leukemic children during steroid and CASP administration, e.g. 0.0 to 2.4%. In contrast, the percentage of patients suffering a thromboembolic event was higher in the PRED group: 10.4% of these children suffered a venous thrombosis during induction therapy. Patients were recruited from the same living population and had a similar rate of central venous lines.

Within both patient groups the rate of established prothrombotic risk factors was found within the prevalence rate reported for healthy Caucasian individuals, and the recorded positive family history of thrombosis in the patients investigated was less than 1%. Whereas 46.5% of the PRED treated children with at least one prothrombotic risk factor (FV G1691A, FII G20210A, protein S, protein C, and antithrombin deficiency, or elevated level of Lp(a)) suffered a symptomatic thromboembolism, no child in the DEXA group with such a risk factor suffered a symptomatic thromboembolism during ALL induction therapy. The reported nine-year-old girl in the DEXA group suffered cerebral venous thromboembolism based on venous hypoplasia of the affected sinus. In this patient, no association with further acquired risk factors, e.g. a decrease of coagulation inhibitors, could be identified. The cerebral venous thromboembolism which had occurred during reinduction in the DEXA group is within the rate reported in the PRED group (application of dexamethasone during reinduction therapy). Thus, the administration of DEXA instead of PRED during induction therapy seems to be mainly responsible for the reduced rate of symptomatic thromboembolism in the children investigated.

Besides the involvement of the above mentioned genetic risk factors of thrombophilic, additional factors such as endothelial cell injury or an acquired coagulation imbalance, commonly described during combined steroid and CASP administration in childhood leukemia, may function as trigger mechanisms for thromboembolic manifestation during
childhood ALL in children treated according to the BFM 90/95 study protocols. In the present survey the possibility of further hemostatic alterations within the BFM protocols with the change of one drug only is described for the first time: Data presented here from the subgroup analysis clearly show that, during the time of similar maximum CASP activity, fibrinogen concentrations as well as activities of antithrombin (major changes), plasminogen and free protein S antigen (minor changes) were significantly reduced in the PRED treated children. Moreover, correlation analysis between CASP activities and levels of coagulation factors revealed no significant results. Hence, a direct relationship between CASP based on CASP administration of 5000 U/m² and levels of coagulation factors as well as the frequency of thrombotic events is unlikely.

Glucocorticoids administered along with CASP in the children investigated have a number of inhibitory effects during inflammatory reactions. Early effects are inhibition of developing edema, capillary dilatation, deposition of fibrin, and migration of leukocytes, whereas late effects are inhibition of capillary proliferation, proliferation of fibroblasts, and deposition of collagen. Moreover, glucocorticoids have a protective effect on the integrity of cell membranes and reduce the synthesis of prostaglandins and thromboxane through inhibition of arachidonic acid release from phospholipids. On the one hand, it has to be discussed that dexamethasone has a stronger glucocorticoid effect than prednisone, which is why these aspects may explain a more protective role of dexamethasone concerning the development of thrombotic events. However, it remains still unclear in how far these mechanisms may alter levels of coagulation factors and platelet function leading to thromboembolism. The data presented here are in agreement with previously published findings measured during the course of BFM reinduction therapy (90/95 protocols: DEXA and CASP). In the latter study values measured for fibrinogen, antithrombin, and plasminogen were found within the range reported in the present study (DEXA group), in which moderate acquired deficiency states of the coagulation proteins investigated were observed only in the minority of cases. Thus, on the other hand, we speculate that the decreased values of plasmatic coagulation
proteins found in the PRED group during ALL induction therapy are possibly due to a more pronounced synergistic effect of CASP and PRED in lowering hepatic protein synthesis compared with CASP and DEXA. Further studies, however, are necessary to clarify this issue.

Pui et al. have demonstrated in 1985 and 1987\textsuperscript{7,16} that an altered von Willebrand factor molecule was found in leukemic children treated with CASP and PRED. Therefore we have included the determination of von Willebrand factor antigen in the study presented here. VWF was significantly higher in children treated with DEXA, but no association was found with thromboembolic events in the patients presented here. Factor VIIIC coagulant activity was investigated only in children with symptomatic thrombosis from samples obtained prior to the thromboembolic onset: Neither in the PRED treated children nor in the DEXA group elevated factor VIIIC levels above 120% of normal were found. This observation is in agreement with data shown by Pui et al. 1985.\textsuperscript{7} We therefore conclude that increased values of factor VIIIC measured from samples prior to the onset of symptomatic thrombosis do not play an important role in the etiology of chemotherapy-induced vascular accidents in German children treated according the BFM-protocols.

Whereas no conclusion can be drawn so far with respect to the overall leukemic event-free survival in patients treated according to the different German BFM 90/95 and BFM 2000 treatment protocols, data of this multicentre survey suggest that the old problem of thromboembolic complications in ALL patients should be revisited: Evidence is given that the combination of inherited prothrombotic risk factors with acquired deficiencies of one or more proteins regulating thrombin and fibrin formation\textsuperscript{5} are responsible for the development of thromboembolic events in leukemic children treated with CASP and prednisone. The use of dexamethasone instead of prednisone, concomitantly administered with CASP in leukemic patients of the same living population treated according to BFM-adapted protocols during induction therapy, significantly reduced the symptomatic onset of venous thromboembolism in the children investigated.
With respect to symptomatic thromboembolism, further studies are recommended to clarify the possible protective effect of dexamethasone during ALL induction therapy.

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REFERENCES


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Table. Median (range) values of plasmatic coagulation and fibrinolytic factors during maximum CASP activity (days 27-33) in children during induction therapy: BFM 90/95 protocol (PRED group) versus BFM 2000 protocol (DEXA group).
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