Impaired dexamethasone-related increase of anticoagulants is associated with the development of osteonecrosis in childhood acute lymphoblastic leukemia

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ABSTRACT

Coagulation alterations may be involved in osteonecrosis in childhood acute lymphoblastic leukemia. Retrospectively, we evaluated the available coagulation parameters at diagnosis and during induction treatment of 161 acute lymphoblastic leukemia patients: 24 with symptomatic osteonecrosis (median age: 13.8 years, range 4.0–17.2) and 137 without osteonecrosis (median age: 4.9 years, range 1.0-16.7). Coagulation parameters of both groups were similar at diagnosis. After four weeks of treatment including dexamethasone, levels of antithrombin and protein S were significantly less in osteonecrosis-positive than in osteonecrosis-negative patients. Subsequently, after four doses of asparaginase and tapering dexamethasone, these coagulation parameters equally decreased in both groups. Consequently, nadirs of antithrombin and protein S were significantly lower in osteonecrosis-positive than in osteonecrosis-negative patients, even reaching levels below lower normal limits in the osteonecrosis-positive group. A reduced dexamethasone related increase of antithrombin and protein S, and subsequent decline below normal levels after introduction of asparaginase, may result in a hypercoagulable state, contributing to development of symptomatic osteonecrosis.

Key words: osteonecrosis, hypercoagulability, antithrombin, protein S, acute lymphoblastic leukemia.

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Introduction

Because the cure-rate of acute lymphoblastic leukemia (ALL) is high, attention has shifted to its side-effects during and after therapy. One of these serious complications is osteonecrosis (ON), which is caused by impairment of microcirculation serving a segment of bone that has a poor collateral circulation and insufficient venous drainage.[1,2]

Hypercoagulability may play a role in the etiology of ON in childhood ALL.[3-5] Dexamethasone and asparaginase, important drugs in the induction treatment of childhood ALL, can influence the coagulation system.[6-10] Corticosteroids increase most coagulation-protein concentrations, whereas asparaginase can reduce the synthesis of coagulation factors and inhibitors. Micro-thrombi resulting from an imbalance between procoagulant and anticoagulant processes in patients with Legg-Calvé-Perthes disease have been shown to play an important etiological role in the development of ON.[11-13] Little information is available on the role of coagulation dysregulation in the pathogenesis of ON in childhood ALL.[3,4,14]

Therefore, the main objective of this study is to investigate whether induction-therapy-related alterations in coagulation are associated with the development of symptomatic ON during childhood ALL. For this reason procoagulant factors, anticoagulant factors, parameters of thrombin generation, and parameters of fibrinolysis were evaluated.

Design and Methods

Patients

Differences in coagulation parameters between childhood ALL patients with and without symptomatic ON were studied in a retrospective analysis. Between 1997 and 2004, 174 patients received induction therapy according to the Dutch Childhood Oncology Group(DCOG)-ALL9 treatment protocol. The purpose of this dexamethasone-based protocol was to reproduce the results of the DCOG-ALL6 treatment protocol in a larger cohort of Dutch ALL patients; it included induction therapy as shown in Figure 1.[15] Since patients received four weeks of dexamethasone prior to any asparaginase exposure, we had a unique opportunity to evaluate the effect of the separate agents on coagulation parameters.

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Patients were treated according to the high-risk protocol based on white-cell count ≥50×10^9, T-cell immunophenotype, mediastinal mass, involvement of the central nervous system, infiltration of the testes, and t(9;22) or 11q23 rearrangements; all other patients received the non-high-risk protocol. Cumulative doses of the chemotherapeutic agents used in the DCOG-ALL9 protocol have been previously reported.[16] In this protocol newly diagnosed ALL patients older than 365 days and younger than 19 years were included, with exception of mature B-cell ALL patients. Coagulation parameters, which were routinely measured during induction therapy to monitor asparaginase toxicity, were available for 161 patients (24 developed symptomatic ON). No patient received plasma or cryoprecipitate during the study period.

Clinical data (age, gender, immunophenotype of leukemia) and data on thrombotic events during intensification and maintenance phase were collected from the medical records. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam and written informed consent was obtained from all participants and/or their parents.

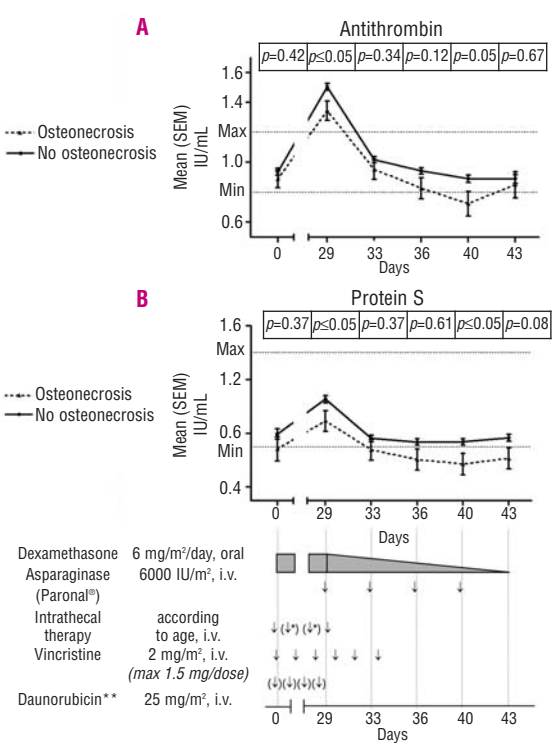
Methods

Patients were considered ON-positive if they had developed symptomatic ON during treatment or within one year after cessation of treatment. For this study we aimed to investigate clinical relevant symptomatic ON.[17] Symptomatic ON was defined as persistent pain in the arms or legs not associated with recent vincristine administration, in combination with typical findings on magnetic resonance imaging(MRI).[18]

Peripheral-blood samples were taken at diagnosis and at five time points (days 29, 33, 36, 40 and 43) during induction therapy of ALL (Figure 1). On days 29, 33, 36 and 40, peripheral-blood samples were taken immediately before each asparaginase infusion. Using sample tubes containing a fixed amount of citrate, we determined procoagulant factors(fibrinogen and factor II,V,VII,IX,X), anticoagulant factors(antithrombin, protein C and protein S), parameters of thrombin generation (prothrombin fragment 1+2(F1+2) and thrombin-antithrombin complex(TAT)) and parameters of fibrinolysis(a2-antiplasmin (a2AP), plasminogen, plasmin-a2AP complex(PAP), D-dimers). Samples were chilled immediately on ice and centrifuged 30 mins. at 20,000 rpm at 4°C. The supernatant was withdrawn and stored until the time of analysis at -80°C.

Coagulation assays

Fibrinogen was determined using the Claus method[19] (Dade® Thrombin Reagent; Dade Behring GmbH, Marburg, Germany). Factor II,V,VII,IX and X were assessed using factor-deficient plasma and standard one-stage factor assays (Coagulation Factor II,VII and X, Coagulation Factor V Deficient Plasma and Coagulation Factor IX; Dade Behring GmbH, Marburg, Germany). The anticoagulants antithrombin and protein C activity were measured using Berichrom Antithrombin III(A) and Berichrom Protein C(Dade Behring GmbH, Marburg, Germany). Total and free protein S were determined using enzyme-linked immunosorbent assays(ELISA)(Asserachrom® Total Protein S and Asserachrom® Free Protein S; Diagnostica Stago, Asnieres, France). Plasminogen and α2-AP were measured using Berichrom A2-Antiplasmin and Berichrom Plasminogen(Dade Behring GmbH, Marburg, Germany). PAP was measured using PAP micro ELISA(Dade Behring GmbH, Marburg, Germany).[20] D-dimer levels were measured with an immunoturbidimetric assay(Auto Dimer®; Trinity Biotech plc, Bray, Ireland). The markers of endogenous thrombin generation F1+2 and TAT were measured using ELISA(Enzygnost F1+2 and Enzygnost TAT; Dade Behring GmbH, Marburg, Germany).[21]



*Only in case of central nervous system involvement
**Daunorubicin was only given in patients treated according to the high risk protocol

Figure 1. Antithrombin (A) and protein S (B) at diagnosis and during Dutch Childhood Oncology Group (DCOG)-ALL9 induction treatment in childhood patients who did and did not develop symptomatic osteonecrosis. SEM: standard error of the mean; i.v., intra venous. Normal reference ranges are marked with dotted horizontal lines; antithrombin: 0.8-1.2 IU/mL and protein S: 0.7-1.4 IU/mL. *p* values indicate differences between patients with and without symptomatic osteonecrosis at each moment (mixed-model analysis of repeated measures).

Data analysis

The Mann-Whitney U-test and the χ^2-test/Fisher-exact test were used to compare patients' characteristics between the ON-positive and ON-negative groups. Coagulation parameters of the ON-positive and ON-negative patients were compared with the non-parametric Mann-Whitney U-test. These statistical analyses were carried out with SPSS for Windows version 11.0.1

Table 1. Clinical characteristics of patients with and without symptomatic osteonecrosis.

Clinical data	*Patients with osteonecrosis*	*Patients without osteonecrosis*	*p value*
Patients at diagnosis (n=)	24	137	-
Gender (male/female)	12 / 12	81 / 56	0.40
Age at ALL diagnosis median (range) in years	13.8 (4.0–17.2)	4.9 (1.0–16.7)	<0.01
Age at diagnosis of osteonecrosis median (range) in years	16.1 (6.0–18.7)	N.A.	-
Immunophenotype			
precursor-B	20 (83%)	116 (85%)	0.77
T	4 (17%)	21 (15%)	
Risk-group stratification			
Non-high risk	18 (75%)	93 (68%)	0.63
High risk	6 (25%)	44 (32%)	

ALL: acute lymphoblastic leukemia; N.A.: not applicable.
p value: Mann-Whitney U-test/χ^2 test/Fisher exact test.

(SPSS Inc., Chicago, IL, USA).

Repeated-measurements analysis (SAS PROC MIXED; SAS Institute Inc., Cary, North Carolina, USA) was used to confirm differences in antithrombin, protein C and protein S between both groups. To analyze differences between the ON-positive and the ON-negative groups in trends over time of antithrombin, protein C and protein S, the model defined by the variables *time*, *ON-group* and the interaction variable *time*ON-group* were applied (Figure 1). We used an unstructured repeated covariance type. Differences between ON-positive and ON-negative patients at each moment were estimated using a model without intercept defined by the interaction variable *ON-group*time*. The same model was used for evaluation of the slopes of the curves of the anticoagulants over time. *p* values under 0.05 (two-sided) were considered statistically significant.

Results and Discussion

Patients' characteristics

Clinical characteristics of ON-positive and ON-negative patients are summarized in Table 1. No differences were found between ON-positive and ON-negative patients in gender, immunophenotype of leukemia and risk-group stratification. As expected, the median age at diagnosis of ALL was significantly higher in ON-positive than in ON-negative patients. ON was confirmed by MRI in all cases. All but one of the ON-positive patients were diagnosed with ON before stop of treatment; the patient diagnosed after stop of treatment already had symptoms during treatment, which were thought to be due to vincristine. Only after cessation of treatment did it become apparent that the patient had ON and this was confirmed by a delayed MRI.

At a later stage during treatment, i.e. intensification and maintenance treatment of the DCOG-ALL9 schedule, 3 out of 24 ON-positive patients (12.5%) endured a thrombotic event, as compared to 2 out of 137 (1.5%) ON-negative patients (χ^2-test, p=0.02). The thrombotic events included one transverse sinus thrombosis, one pulmonary embolism and a thrombus of the brachiocephalic vein possibly related to the presence of an implantable venous access port (Port-A-Cath) in the ON-positive patients. In the group of ON-negative patients, one transverse sinus thrombosis and one catheter related thrombosis in the upper venous system of the arm occurred.

Coagulation parameters at diagnosis

Values of the coagulation parameters at diagnosis are summarized in Table 2. At diagnosis no significant differences in any of the coagulation parameters between ON-negative patients and ON-positive patients were observed. Values of the anticoagulant factors antithrombin, protein C activity and total protein S were all within normal ranges for both groups (Figure 1).

Coagulation parameters during induction treatment

Results of all coagulation parameters measured during DCOG-ALL9 induction therapy after a four-week treatment with dexamethasone (day 29) are shown in Table 2. Mean values of the anticoagulants antithrombin and total protein S after four weeks of induction treatment with dexamethasone at day 29 and during tapering of dexamethasone and administration of four doses asparaginase (day 33-43) are shown in Figure 1. Values of antithrombin and total protein S, but not of protein C, were significantly lower in ON-positive patients than in ON-negative patients after four weeks of dexamethasone administration (p<0.05 and p<0.05). Mixed-model analysis of repeated measures showed a significant decrease in ON-positive patients of antithrombin (p<0.001) and total protein S (p<0.001) during days 29-43 and also in ON-negative patients there was a significant decrease of antithrombin (p<0.001) and protein S (p<0.001) from day 29-43 (Figure 1). This decrease of AT and total protein S during tapering of dexamethasone and administration of four doses of asparaginase was equal in both ON-positive and ON-negative patients, which resulted in a decline below lower normal limits of antithrombin and protein S in ON-positive patients but not in ON-negative patients.

Factor X was different between ON-positive and ON-negative patients at day 29, but absolute levels stayed within the normal range in both groups at all time points. Dexamethasone-induced or asparaginase-induced differences in any of the other coagulation parameters between ON-negative and ON-positive patients were not found at any time point during induction treatment.

Osteonecrosis is a disabling complication which may occur during ALL treatment. Although several etiological factors have been suggested, the pathophysiology of ON is not entirely understood. It has been suggested that a deviation of the coagulation system is one of the predisposing factors, but until now only a few studies

Table 2. Coagulation parameters at diagnosis and after four weeks induction therapy with dexamethasone of pediatric patients with acute lymphoblastic leukemia who did and did not develop osteonecrosis.

Coagulation variable	*Normal ranges*	*Diagnosis*			*Diagnosis*			*After 4 weeks dexamethasone*		*After 4 weeks dexamethasone*	
		ON-pos. (N=)	*ON-pos. Median (range)*	*ON-neg. (N=)*	*ON-neg. Median (range)*	*p*	*ON-pos. (N=)*	*ON-pos. Median (range)*	*ON-neg. (N=)*	*ON-neg. Median (range)*	*p*
PT (sec)	10.9-13.3	21	14.0 (10.0-21.5)	107	13.1(10.0-29.0)	0.71					
APTT (sec)	28-39	23	34 (19-48)	136	34 (20-56)	0.77					
Fibrinogen (g/L)	1.5-3.6	24	3.2 (1.7-4.7)	137	3.0 (0.8-7.8)	0.69	9	0.9 (0.1-2.1)	78	1.3 (0.1-3.9)	0.11
Antithrombin (IU/mL)	0.80-1.20	13	0.93 (0.51-1.20)	84	0.94 (0.47-1.44)	0.41	9	1.29 (0.96-1.68)	78	1.47 (1.03-2.02)	<0.05
ProtCact (IU/mL)	0.70-1.40	9	0.68 (0.53-1.09)	59	0.72 (0.37-1.29)	0.66	9	1.56 (0.94-2.96)	78	1.84 (0.84-3.29)	0.09
ProtSfree (IU/mL)	0.76-1.28	3	0.51 (0.29-0.64)	26	0.58 (0.21-1.07)	0.45	9	0.89 (0.63-1.50)	78	1.00 (0.11-1.54)	0.17
ProtStotal (IU/mL)	0.70-1.40	7	0.66 (0.56-1.08)	33	0.74 (0.33-1.49)	0.46	9	0.81 (0.64-1.53)	78	1.03 (0.28-1.53)	<0.05
Plasminogen (IU/mL)	0.85-1.20	6	0.86 (0.76-1.29)	33	1.03 (0.43-1.49)	0.52	9	1.04 (0.67-1.61)	76	1.21 (0.80-2.34)	0.08
a2AP (IU/mL)	0.80-1.20	6	1.04 (0.92-1.20)	33	1.10 (0.42-1.35)	0.21	9	1.27 (0.97-1.65)	76	1.41 (0.95-2.82)	0.06
D-dimers (mg/L)	0-0.25	13	0.33 (0.10-3.21)	59	0.40 (0.03-4.77)	0.11	7	0.16 (0.07-0.47)	62	0.11 (0.02-0.64)	0.20
Factor II (IU/mL)	0.60-1.40	6	0.52 (0.49-1.33)	33	0.70 (0.24-1.12)	0.53	9	1.21 (0.47-1.39)	78	1.31 (0.12-2.07)	0.13
Factor V (IU/mL)	0.50-1.50	6	1.15 (0.38-1.61)	35	0.86 (0.31-1.89)	0.41	9	1.39 (0.71-1.84)	78	1.35 (0.81-2.40)	0.67
Factor VII (IU/mL)	0.60-1.40	6	0.74 (0.30-0.80)	33	0.65 (0.33-0.92)	0.45	9	0.97 (0.33-1.53)	78	1.12 (0.54-1.98)	0.16
Factor IX (IU/mL)	0.60-1.50	6	1.23 (0.91-1.87)	33	1.16 (0.61-2.08)	0.47	9	1.62 (1.03-2.01)	78	1.79 (0.41-2.66)	0.41
Factor X (IU/mL)	0.60-1.40	6	0.77 (0.57-0.94)	33	1.05 (0.14-1.62)	0.07	9	1.22 (0.67-1.66)	78	1.56 (0.62-2.52)	<0.05
TAT (ug/L)	1.0-4.1	6	9.3 (4.0-56.7)	33	15.1 (3.6-60.0)	0.18	9	6.0 (2.6-22.0)	74	4.5 (1.6-414.0)	0.57
F1+2 (pmol/L)	69-229	6	549 (104-1500)	33	576 (145-1500)	0.78	3	343 (251-1339)	27	190 (70-521)	<0.05
F1+2 (nmol/L)	0.44-1.11						6	1.52 (0.99-5.36)	49	1.08 (0.49-20.54)	0.07
PAP (ug/L)	120-700	6	838 (288-1247)	33	634 (317-2318)	0.63	9	306 (3-1403)	74	251 (51-1023)	0.78

ON: osteonecrosis. p values: Mann-Whitney U-test.

have been performed on coagulation dysregulation in the pathogenesis of ON in childhood.[11-13] In 1989, Hanada *et al*. suggested that L-asparaginase induced coagulopathy in a child with ALL related osteonecrosis.[5] A study investigating the prevalence of hereditary prothrombotic risk factors such as factor V Leiden, the prothrombin 20210G>A polymorphism and the methylene tetrahydrofolate reductase 677C>T variant in a group of 24 children who developed ON during treatment for various types of cancer, including 16 cases of ALL, did not identify an increased prevalence of these hypercoagulable state mutations.[3] In a previous study, we showed that the number of kringle-IV repeats in the Apo(a) gene and lipoprotein(a) levels did not contribute to an increased risk of symptomatic ON during childhood ALL.[4] Recently PAI-1 genetic variation was described as contributing to the risk of osteonecrosis in children with ALL.[14] The present study is the first to investigate the influence of coagulation disturbance during induction treatment, as measured by procoagulant factors, anticoagulant factors, parameters of thrombin generation and parameters of fibrinolysis, on the development of symptomatic ON in a single-center cohort of children with ALL, treated according to one risk-stratified treatment protocol.

We found no differences in coagulation parameters at diagnosis between the ALL patients who developed ON and patients who did not. This suggests that there are no important pre-existent patient-specific or leukemia-related coagulation aberrations that play a role in the pathogenesis of ON in childhood ALL.

We suggest that asparaginase plays an important role in the development of symptomatic ON in ALL patients, illustrated by the significant decrease of antithrombin and protein S in all patients after the introduction of this drug. However, our results show that the preceding administration of dexamethasone might play an even more discriminative role for developing ON. ON-positive ALL patients showed a less impressive and significantly different increase in antithrombin and protein S after administration of dexamethasone. A subsequent decrease of antithrombin and protein S levels below the lower limit of normal in the ON-positive patients did not become manifest until asparaginase administration. This may indicate a therapy-induced hypercoagulable state in this subgroup of ALL patients, contributing to the development of ON. As total protein S levels were determined only in a sufficient number of patients, it remains unknown whether free protein S levels were also reduced, which may be more clinically relevant.

Previous studies showed that ON is a serious complication of childhood ALL treatment, especially in teenagers.[22,23] The higher incidence of ON in older children has not yet been explained. The majority of the ON-positive patients in our study were above the age of ten years (79%). Although in the total group of patients high levels of antithrombin and protein S after four weeks of dexamethasone treatment were found, subgroup analyses showed a significantly different increase in levels of these anticoagulants in children older than ten years of age as compared to the younger ALL patients.[24] As our group of ON-positive patients was significantly older than the group of ON-negative patients, we suggest that age-related differences in dexamethasone-induced changes in the coagulation system may contribute to this higher incidence of ON at an older age. Considering the limitations of our retrospective

study design, future prospective studies are necessary to validate the contribution of these age-related coagulation aberrations in the development of ON and the role of free protein S levels has to be established.

In conclusion, the present study indicates that a hypercoagulable state may result from a lower dexamethasone-related increase of antithrombin and protein S and the subsequent decline of these anticoagulants below normal levels after introduction of asparaginase. This therapy-induced hypercoagulable state may contribute to the development of symptomatic ON, especially in teenagers.

Authorship and Disclosures

MLtW performed statistical analysis and wrote the manuscript; IMA supervised the statistical analysis and wrote the manuscript; RP designed research, supervised the statistical analysis and wrote the manuscript; MMvdH-E designed research, supervised the statistical analysis and wrote the manuscript. The authors reported no potential conflicts of interest.

References

1. Glimcher MJ, Kenzora JE. The biology of osteonecrosis of the human femoral head and its clinical implications: II. The pathological changes in the femoral head as an organ and in the hip joint. Clin Orthop Relat Res 1979:283-312.
2. Jones JP Jr. Intravascular coagulation and osteonecrosis. Clin Orthop Relat Res 1992:41-53.
3. Kechli AM, Wilimas JA, Pui CH, Park VM, Tonkel S, Deitcher SR. Factor V Leiden and other hypercoagulable state mutations are not associated with osteonecrosis during or after treatment for pediatric malignancy. J Pediatr 1999;134:310-4.
4. van Beek RD, Bezemer DD, Meijerink JP, de Muinck Keizer-Schrama SM, Haas OA, Te Winkel L, et al. Repeats in the kringle IV encoding domains in the Apo(a) gene and serum lipoprotein(a) level do not contribute to the risk for avascular necrosis of the bone (AVN) in pediatric acute lymphoblastic leukemia. Leukemia 2006;20:879-80.
5. Hanada T, Horigome Y, Inudoh M, Takita H. Osteonecrosis of vertebrae in a child with acute lymphocytic leukaemia during L-asparaginase therapy. Eur J Pediatr 1989;149:162-3.
6. Appel IM, Hop WC, Pieters R. Changes in hypercoagulability by asparaginase: a randomized study between two asparaginases. Blood Coagul Fibrinolysis 2006;17:139-46.
7. Athale UH, Chan AK. Thrombosis in children with acute lymphoblastic leukemia. Part II. Pathogenesis of thrombosis in children with acute lymphoblastic leukemia: effects of the disease and therapy. Thromb Res 2003;111:199-212.
8. Caruso V, Iacoviello L, Di Castelnuovo A, Storti S, Mariani G, de Gaetano G, et al. Thrombotic complications in childhood acute lymphoblastic leukemia: a meta-analysis of 17 prospective studies comprising 1752 pediatric patients. Blood 2006; 108:2216-22.
9. Mitchell LG, Halton JM, Vegh PA, Barr RD, Venneri T, Pai KM, et al. Effect of disease and chemotherapy on hemostasis in children with acute lymphoid leukemia. Am J Pediatr Hematol Oncol 1994;16:120-6.
10. Nowak-Gottl U, Ahlke E, Fleischhack G, Schwabe D, Schobess R, Schumann C, et al. Thromboembolic events in children with acute lymphoblastic leukemia (BFM protocols): prednisone versus dexamethasone administration. Blood 2003; 101:2529-33.
11. Szepesi K, Posan E, Harsfalvi J, Ajzner E, Szucs G, Gaspar L, et al. The most severe forms of Perthes' disease associated with the homozygous factor V Leiden mutation. J Bone Joint Surg Br 2004;86:426-9.
12. Balasa VV, Gruppo RA, Glueck CJ, Wang P, Roy DR, Wall EJ, et al. Legg-Calve-Perthes disease and thrombophilia. J Bone Joint Surg Am 2004; 86:2642-7.
13. Glueck CJ, Fontaine RN, Gruppo R, Stroop D, Sieve-Smith L, Tracy T, et al. The plasminogen activator inhibitor-1 gene, hypofibrinolysis, and osteonecrosis. Clin Orthop Relat Res 1999:133-46.
14. French D, Hamilton LH, Mattano LA Jr, Sather HN, Devidas M, Nachman JB, et al. A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood 2008;111:4496-9.
15. Veerman AJ, Hahlen K, Kamps WA, Van Leeuwen EF, De Vaan GA, Solbu G, et al. High cure rate with a moderately intensive treatment regimen in non-high-risk childhood acute lymphoblastic leukemia. Results of protocol ALL VI from the Dutch Childhood Leukemia Study Group. J Clin Oncol 1996;14:911-8.
16. van der Sluis IM, van den Heuvel-Eibrink MM, Hahlen K, Krenning EP, de Muinck Keizer-Schrama SM. Altered bone mineral density and body composition, and increased fracture risk in childhood acute lymphoblastic leukemia. J Pediatr 2002; 141:204-10.
17. Ribeiro RC, Fletcher BD, Kennedy W, Harrison PL, Neel MD, Kaste SC, et al. Magnetic resonance imaging detection of avascular necrosis of the bone in children receiving intensive prednisone therapy for acute lymphoblastic leukemia or non-Hodgkin lymphoma. Leukemia 2001;15:891-7.
18. Robinson HJ Jr, Hartleben PD, Lund G, Schreiman J. Evaluation of magnetic resonance imaging in the diagnosis of osteonecrosis of the femoral head. Accuracy compared with radiographs, core biopsy, and intraosseous pressure measurements. J Bone Joint Surg Am 1989; 71:650-63.
19. Clauss A. [Rapid physiological coagulation method in determination of fibrinogen.]. Acta Haematol 1957; 17:237-46.
20. Holvoet P, de Boer A, Verstreken M, Collen D. An enzyme-linked immunosorbent assay (ELISA) for the measurement of plasmin-alpha 2-antiplasmin complex in human plasma--application to the detection of in vivo activation of the fibrinolytic system. Thromb Haemost 1986; 56:124-7.
21. Ries M, Klinge J, Rauch R. Age-related reference values for activation markers of the coagulation and fibrinolytic systems in children. Thromb Res 1997;85:341-4.
22. Arico M, Boccalatte MF, Silvestri D, Barisone E, Messina C, Chiesa R, et al. Osteonecrosis: An emerging complication of intensive chemotherapy for childhood acute lymphoblastic leukemia. Haematologica 2003;88: 747-53.
23. Nachman JB. Adolescents with acute lymphoblastic leukemia: a new "age". Rev Clin Exp Hematol 2003; 7:261-9.
24. Appel IM, van Kessel-Bakvis C, Stigter R, Pieters R. Influence of two different regimens of concomitant treatment with asparaginase and dexamethasone on hemostasis in childhood acute lymphoblastic leukemia. Leukemia 2007;21:2377-80.