Osteonecrosis in children with acute lymphoblastic leukemia

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ABSTRACT

The morbidity and toxicity associated with current intensive treatment protocols for acute lymphoblastic leukemia in childhood become even more important as the vast majority of children can be cured and become long-term survivors. Osteonecrosis is one of the most common therapy-related and debilitating side effects of anti-leukemic treatment and can adversely affect long-term quality of life. Incidence and risk factors vary substantially between study groups and therapeutic regimens. We therefore analyzed 22 clinical trials of childhood acute lymphoblastic leukemia in terms of osteonecrosis incidence and risk factors. Adolescent age is the most significant risk factor, with patients >10 years old at the highest risk. Uncritical modification or even significant reduction of glucocorticoid dosage cannot be recommended at this stage. A novel and innovative approach to reduce osteonecrosis-associated morbidity might be systematic early screening for osteonecrosis by serial magnetic resonance images. However, discriminating patients at risk of functional impairment and debilitating progressive joint disease from asymptomatic patients still remains challenging.

Background

Survival of children with acute lymphoblastic leukemia (ALL) has dramatically improved over the last decades due to the progressive intensification of multi-agent chemotherapy. Currently, more than 90% of children and adolescents can be cured and become long-term survivors.[1,2] Thus, the long-term adverse effects of treatment become increasingly important. Osteonecrosis is one of the most common and debilitating therapy-related side effects of anti-leukemic treatment and can adversely affect long-term quality of life.[3] Incidence (1.6–17.6%) and risk factors for the development of osteonecrosis have been investigated in many studies, but results vary substantially between study groups and therapeutic regimens.[4-9] Adolescence is the most consistently identified and most significant risk factor, with patients >10 years old at the highest risk.[7-11] As this dominates all other therapy-related and patient-specific risk factors, it suggests that the underlying pathophysiology for the development of osteonecrosis likely has to be attributed to age-specific factors ultimately affecting bone morphology, metabolism, and/or nourishment. This may be due, at least in part, to increased end-organ susceptibility caused by a markedly increased growth rate and specific hormonal changes in this period of life.[12]

Current concepts of osteonecrosis pathogenesis

The early events leading to osteonecrosis are poorly understood. Multiple factors for the development of osteonecrosis are discussed, which probably act synergistically in the context of anti-leukemic treatment. All contributing mechanisms finally lead to an imbalance between the actual and the required bone perfusion, which

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may be related to intravascular clotting/embolism (intraluminal obliteration), increased marrow pressure (extraluminal obliteration), and direct blood vessel injury. In addition, the direct toxic effects of chemotherapy on bone marrow and bone cells may disturb bone integrity and contribute to osteonecrosis .[13]

Although the underlying disease and the exposure to damaging agents, such as glucocorticoids (GCs), are of a systemic nature, osteonecrosis predominantly develops in vulnerable areas such as long bone epiphysis and metaphysis (Table 1).

Disrupted blood supply to the bone

Bone is a highly perfused tissue. The blood supply to the endosteal cavity is delivered by the nutrient artery, which enters through the diaphysis and branches into marrow sinusoids, and finally ramifies into small vessels in the cortex. The epiphyseal and metaphyseal vascular zones of prepubertal children are separated by the growth plate, which receives its blood supply only from dia- and epiphyseal vessels and anastomoses in the perichondrium, respectively (Figure 1). Neural, humoral, and hormonal factors contribute to the regulation of vascular resistance, and, thus, influence the blood supply to the bone.

Intraluminal obliteration

Liver-to-bone marrow lipid emboli trigger thrombotic and/or embolic ischemia, resulting in cell damage and subsequent bone marrow edema (BME). This leads to ischemic necrosis of metabolically active/vulnerable regions such as the epiphyses.[14,15] By triggering intravascular coagulation in the intraosseous microcirculation (capillaries and venous sinusoids), increased prothrombotic factors (e.g., thrombin, cholesterol) contribute to the development of osteonecrosis.[16]

Extraluminal obliteration

Intramedullary lipocyte proliferation (compromising the sinusoidal circulation) and osteocyte lipid hypertrophy (e.g., related to GCs or dyslipidemia), proliferation of histiocytes in storage disorders (e.g., Gaucher disease), or bleeding within the bone marrow cause increased intramedullary pressure. Because of the inelasticity of the bone, intraosseous compartment syndrome develops, further reducing intramedullary blood flow and predisposing for hemostasis in the intraosseous blood vessels.[17-19]

The epiphyseal plate of the immature bone during childhood growth provides elasticity to compensate for the increasing intraosseous pressure, while with epiphyseal closure during adolescence, the intramedullary pressure increases and can be passed through to the epiphyseal part of the bone.

Table 1. Distribution pattern of osteonecrosis in children and adolescents with ALL (acute lymphoblastic leukemia) according to published data.

Joints affected	%	References
Shoulder	13-24	7, 39, 43, 46, 62
Elbow	3-15	7, 39, 43, 46, 62
Hip	35-67	3, 7, 8, 10, 33, 34, 39, 43, 46, 62
Knee	45-88	3, 7, 8, 10, 33, 39, 43, 46, 62
Ankle	13-44	7, 8, 10, 34, 39, 43, 46, 62
Multiple joints	29-90	3, 7, 8, 10, 32, 34, 39, 41, 42, 46, 62

Direct blood vessel injury

Disruption of the vascular supply to the bone is a preceding event to glucocorticoid-induced osteonecrosis in a murine model.[20] This is mainly mediated by damaging effects on the endothelial and smooth muscle cells of nutrient arteries and venous vessels, which promote further vascular stasis, ischemia, and arteriopathy.[21]

Altered integrity of bone structure

Longitudinal bone growth occurs by endochondral bone formation, particularly in the growth plates at the proximal and distal ends of long bones, whereas bone growth in width occurs by bone modeling. During remodeling, the bone tissue is continuously turned over. Both osteoclasts and osteoblasts are fundamentally involved in this process and influence bone development during childhood and adolescence.[22,23] During the pubertal growth spurt, particularly, the bone length increases. Furthermore, sexual hormones impact bone (re)modeling and, thus, affect bone strength and mass.[22]

Direct cell toxicity

GCs are reported to induce gradual lipid accumulation within osteocytes, osteocyte death, increased osteocyte apoptosis, suppression of osteoblastic differentiation of marrow stem cells, decreased cell division of osteoblasts near osteonecrosis lesions, and increased mesenchymal stem cell differentiation into lipocytes at the expense of osteogenesis.[14,20,24-29]

Defective bone repair

During revascularization following ischemia, changes occur in the hematopoietic marrow, fatty marrow, and vascular structures. The surrounding bony architecture within the area of infarction becomes weakened by resorption of subchondral dead bone along the reactive interface. The repair process at least temporarily compromises bone mass integrity. Continued cellular stress, mechanical load/weight-bearing stress fractures, collapse of the chondral bony support system, cartilage disintegration, and deformity of articular surfaces may ultimately lead to progressive joint collapse and degenerative joint disease.[30,31]

Osteonecrosis in the context of anti-leukemic treatment

Osteonecrosis has only recently been recognized as one of the most significant toxicities of anti-leukemic treatment (see Tables 2 and 3). This is in stark contrast to historical experience in which osteonecrosis was considered a rare complication of ALL therapy. In 2000, Mattano *et al.* reported on a large retrospective multi-center survey on symptomatic osteonecrosis in children with high-risk ALL treated according to the CCG-1882 protocol between 1989 and 1995.[7] With a cumulative osteonecrosis incidence of 9.3% and orthopedic interventions in 24% of the affected children, this report highlighted, for the first time, osteonecrosis as a serious problem of modern chemotherapy. A trend to better outcome after occur-

rence of osteonecrosis further emphasized the challenge of treating these children with therapy that maximizes cure rates but is associated with unanticipated and – to a certain extent – unacceptable toxicity. Notably, this was chronologically associated with the introduction of dexamethasone for delayed intensification with improved survival rates, particularly in the most affected group of adolescents. A retrospective study on two consecutive DFCI trials reported a slightly lower osteonecrosis incidence (7%) but an even higher rate (30%) of orthopedic interventions,[32] the former speculatively owed to the fact that dexamethasone was only given in DFCI trial 91-01. A magnetic resonance imaging (MRI) screening based prospective study determined a significantly higher osteonecrosis incidence (15.5%) without the impact of steroid dose or dexamethasone administration (see Table 4).[33] This was even exceeded by a prospective study analyzing the Nordic ALL protocols, which reported an osteonecrosis incidence as high as 24%, identified by MRI screening at the end of treatment.[8] As the earlier reports were based on retrospective data collection, the true osteonecrosis incidence was most likely underestimated. However, 6 of the 17 affected patients reported by Ribeiro *et al.*[33] and 16 of the 23 patients reported by Niinimäki *et al.*[8] were only detected by MRI, and the patients remained asymptomatic until the end of the study. Two independent retrospective reports on Berlin-Frankfurt-Muenster (BFM)-based trials with quite similar therapy (AIEOP-ALL 95,[34] ALL-BFM 95[10]) from the late 90s determined a much lower, but almost identical, osteonecrosis incidence of 1.6–1.8%. However, in patients aged ≥10 years, the osteonecrosis incidence was reported to be 8.9% and even higher in those ≥15 years (16.7%).[10] Thus, when comparing these studies, one has to keep in mind that appropriate age groups must be compared, and that there might be a significant difference in the patients' age distribution in each study, which certainly influences the overall incidence of osteonecrosis. In line with this, Mattano *et al.*[7] only evaluated high-risk patients, but young patients usually make up only about one third of the high-risk group. However, the osteonecrosis incidence in these retrospective studies was

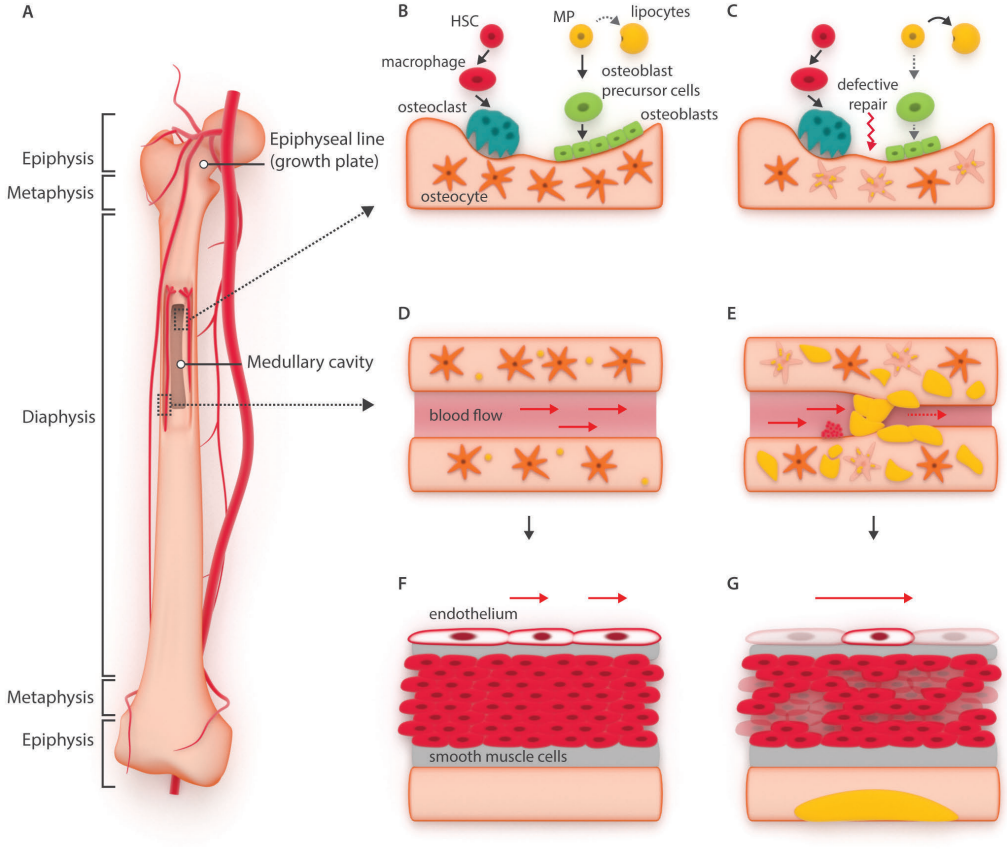

Figure 1. Schematic illustration of osteonecrosis pathogenesis. (A) Blood supply of bone is delivered by the nutrient arteries, which enter in the dia- and epiphysis and branch into marrow sinusoids. The growth plate receives its blood supply from dia- and epiphyseal vessels and anastomoses in the perichondrium, respectively. (B-C) Bone growth occurs by endochondral bone formation and bone modeling. The bone tissue is continuously turning over, with osteoclasts and osteoblasts being fundamentally involved in this process. In the context of osteonecrosis development, osteoblast differentiation from mesenchymal progenitor cells is disturbed by gradual lipid accumulation within osteoblasts and osteocytes and increased cell death, both mainly induced by GCs (glucocorticosteroids), and results in defective bone repair. (D-E) Bone perfusion is disturbed by intraluminal obliteration induced by lipid emboli and intravascular coagulation and extraluminal obliteration induced by intramedullary lipocyte proliferation and lipid hypertrophy. Intraosseous compartment syndrome may develop, increasingly reducing intramedullary blood flow and predisposing for coagulation in the intraosseous blood vessels. (F-G) Direct blood vessel injury and disruption of the vascular supply to the bone is mainly mediated by damaging effects on the endothelial and smooth muscle cells of nutrient arteries and venous vessels. HSC: hematopoietic stem cell; MP: mesenchymal progenitor cell.

probably underestimated as this toxicity was unanticipated, and therefore not listed as a reportable event on the case report forms. Furthermore, the treating physicians in those days were not aware of this toxicity, and a standardized diagnostic approach was lacking. However, if one assumes that osteonecrosis was underreported in these trials, and exposure to dexamethasone increases the risk of osteonecrosis, particularly during delayed intensification, one would expect a much higher incidence of osteonecrosis in the subsequent trial ALL-BFM 2000. Notwithstanding that the overall incidence of osteonecrosis was substantially higher (4.7%)[35] and exceeded that reported in the trial NOPHO ALL-2008 (3.1%),[36] and EORTC-CLG 58951 (2.5%),[37] it still remained lower than that of CCG,[38,39] DFCI,[40,41] DCOG,[42,43] and UK[44,45] trials. Even when comparing only the group of older patients, the incidence (in prospective studies on symptomatic osteonecrosis) is much higher but still varies substantially between the trials (CCG-1961[39] 9.9% 10-15 years, 20% 16-21 years; UKALL 2003[45] 16% 10-15 years, 15% >16 years; NOPHO ALL2008[36] 11% 10-14 years, 6.5% 15-17 years; ALL-BFM 2000[35] 14.5% 10-15 years; DFCI-ALL 00-01[41] 14% 10-18 years). However, a factor which remained consistent throughout all the studies was that older children and adolescents are at a much higher risk of developing osteonecrosis.

Table 2. Overview of retrospective studies reporting incidences and risk factors for symptomatic osteonecrosis in children and adolescents with ALL.

Protocol & recruiting period	Study cohort Data source & no. of participating centers	No of patients (with ON/ALL)	Incidence	Risk factors	Reference	Year
CCG-1882 05/89-06/95	High-risk ALL • 1-9 y with WBC ≥50x10⁹/L • ≥10 y Survey / 56 centers	111/1,409 27 pts with orthop. interv.	9.3% CI mostly confirmed by radiographic imaging	• ***0.9% <10 y vs. 13.5% 10-15 y vs. 18% 16-20 y (S)*** • 12.2% females *vs.* 7.7% males (S) • 10-15 y: 19.2% females *vs.* 9.8% males (S) • 16-20 y: 13.2% females *vs.* 20.7% males • ≥10 y: 16.7% whites *vs.* 3.3% blacks (S) • Slight trend to better outcome after occurrence of ON	Mattano *et al.*[7]	2000
DFCI 87-01 ***DFCI 91-01*** 11/87-12/95	ALL • 0-18 y Records / Single center	13/176 4 pts with orthop. interv.	7% CI confirmed by radiographic imaging	• ***4% <9 y vs. 21% 9-18 y (S)*** • 9% DEX *vs.* 6% PRED (NS) • Sex, risk group, WBC (NS)	Strauss *et al.*[32]	2001
AIEOP-ALL 95 05/95-12/99	Non B-ALL • <18 y Data recall / Multicenter	15/1421	1.6% CI	• ***0.3% 0-5 y vs. 0.7% 6-9 y vs. 7.4% 10-17 y (S)*** • 2.5% female *vs.* 0.7% male (S) • 2.4% SR *vs.* 1.0% IR *vs.* 5.8% HR (S) • Highest risk: Females aged 10-17 y (S)	Arico *et al.*[34]	2003
ALL-BFM 95 01/96-06/00	ALL • 0-18 y Questionnaire / Multicenter	31/1951 13 pts with orthop. interv.	1.8% CI	• ***0.2% <10 y vs. 8.9% ≥10 y (S)*** • 1.3% <15 y *vs.* 16.7% ≥15 y (S) • 0.2% SR *vs.* 2.7% MR (S) • 2.7% MR *vs.* 3.5% HR (NS) • 2.4% female *vs.* 1.4% male (NS)	Burger *et al.*[10]	2005
UKALL97 01/97-12/07	ALL Records / Single center	18/186	9.7% CI	• Age >9 y (S) • 9% female *vs.* 10% male (NS) • 11% DEX *vs.* 3.5% PRED	Elmantaser *et al.*[44]	2010
CoALL-07-03 09/03-12/09	ALL • 1-18 y Records / Single center	22/124 8 pts with orthop. interv.	25% CI confirmed by MRI	• ***13.4% <10 y vs. 52.3% ≥10 y (S)*** • 16.1% females *vs.* 36.2% males (NS) • 8.3% LR *vs.* 39.7% HR (S)	Kuhlen *et al.*[46]	2014
UKALL 2003 10/03-06/11	ALL • 1-24 y Records & questionnaire / Multicenter	153/3.126 30 pts with orthop. interv.	5%	• ***0.7% <10 y vs. 16% 10-15 y vs. 15% >16 y (S)*** • Ethnic group, sex, number of delayed intensifications (NS)	Amin *et al.* (abstr.)[45]	2015
ANZCHOG 8 2002-11	ALL, LBL • Children & adolescents Records / Single center	18/251	7% CI confirmed by MRI	• 29% >10 y (S) • 3.4% SR *vs.* 7.5% MR *vs.* 13.8% VHR (NS) • 5.2% males *vs.* 11.2% females (NS)	Padhye *et al.*[62]	2016
DFCI 05-001 2005-11	ALL • 1-18 y Cohort study	65/730	8.9%	• 3.3% in Hispanic *vs.* 10.3% in non-Hispanic (S)	Kahn *et al.* (abstr.)[40]	2015

PRED: prednisolone; DEX: dexamethasone; CI: cumulative incidence; S: significant: NS: not significant; y: years; Ind: Induction; Intensif: intensification; Maint: maintenance; Cont: continuation; Reind: reinduction; Postrem: postremission; Reintensif: reintensification; Cons: consolidation. ALL : acute lymphoblastic leukemia; ON: osteonecrosis; WBC : white blood count; MRI : magnetic resonance imaging; B-ALL: B cell acute lymphoblastic leukemia; LBL: lymphoblastic lymphoma; pts: patients; orthop. interv.: orthopedic interventions; HR: high-risk; SR: standard-risk; MR: medium risk; IR: intermediate-risk; VHR: very high-risk; LR: low-risk.

It may be speculated that these differences in osteonecrosis incidence may be due to reporting bias, incompleteness of data, and different methods of analysis, and might further be substantially influenced by treatment related or non-treatment related risk factors.

Risk factors

As osteonecrosis seems to be a particularly predominant problem in children and adolescents diagnosed with acute lymphoblastic leukemia, leukemia itself might contribute to the development of osteonecrosis. Lymphoblasts are known to have bone-resorbing effects. However, neither areas of leukemic infiltration of bone[26] nor white blood count at diagnosis[32,33,46] and immunophenotype (T- *versus* B-cell leukemia)[38,46] are associated with osteonecrosis risk.

Treatment related risk factors

Glucocorticoids

GCs are major contributors to the development of osteonecrosis, with the cumulative dose of received GCs correlating with the risk of osteonecrosis (see Table 5).[3,47] In study AALL02[32,38] an excess risk of osteonecrosis was found in older patients with dexamethasone at 10 mg/m^2/d x 14 days (24%) *versus* prednisone at 60 mg/m^2/d x 28 days (16%). Most studies[3,5,32,37,48] report no obviously increased risk of osteonecrosis with the administration of dexamethasone compared to prednisone, even in the risk group of older patients (for example in ALL-BFM 2000,[35] the osteonecrosis incidence in patients treated with dexamethasone was 14% *versus* 19% with prednisone). To make the different trials immediately comparable, many authors calculated the equipotent anti-inflammatory doses

Table 3. Overview of prospective studies reporting incidences and risk factors for symptomatic osteonecrosis in children and adolescents with ALL.

Protocol & recruiting period	Study cohort No. of participating centers	No of patients (with ON/ALL)	Incidence	Risk factors	Reference	Year
ALL97 ALL97/99 04/97-06/02	ALL • 1-18 y Multicenter	15/1603	1% NCI grade 3 / 4	• Older age, female sex (S) • DEX *vs.* PRED (NS)	Mitchell *et al.*[48]	2005
DCOG-ALL9 01/97-11/04	ALL Multicenter	38/694 7 pts with orthop. interv.	6.1% CI confirmed by MRI	• Age (S) • Female sex (S) • NHR *vs.* HR (NS)	te Winkel *et al.*[43]	2011
CCG-1961 09/96-05/02	High-risk ALL • 1-21 y • WBC ≥50x10^9/L • ≥10 y Multicenter	143/2056 62 pts with orthop. interv.	7.7% CI confirmed by MRI	• ***1.0% 1-9 y vs. 9.9% 10-15 y vs. 20.0% 16-21 y (S)*** • 15.7% female *vs.* 9.3% male aged 10-21 y (S) • 8.7% alternate-week *vs.* 17.0% continuous DEX aged ≥10 y during delayed-intensification (S) • >10 y: 5-EFS 85.8% with *vs.* 68.2% without ON, in males and females (S)	Mattano *et al.*[39]	2012
DFCI-ALL 00-01 09/00-12/04	ALL • 1-18 y Multicenter	23/408	6%	• ***3.5% <10 y vs. 14% 10-18 y (S)*** • 5% PRED *vs.* 23% DEX aged 10-18 y (S)	Vrooman *et al.*[41]	2013
EORTC-CLG 58951 12/98-08/08	ALL • <18 y Multicenter	49/1.947	2.5%	• 2.5% DEX *vs.* 2.6% PRED (NS)	Domenech *et al.*[37]	2014
DCOG-ALL9 01/97-11/04	ALL • 4-18 y	30/466	6.4% confirmed by MRI	• lower mean bone mineral density (BMD) of the lumbar spine (LS) and total body (TB) at cessation of treatment (S) • steeper BMDLS and BMDTB decline in pts with ON during follow-up (S)	den Hoed *et al.*[42]	2015
COG-AALL043 01/07-07/14	T-ALL • 1-30 y Multicenter	69/1,155	8% CI imaging confirmed	• ***2.6% 1-9 y vs. 14.6% ≥10 y CI (S)*** • 5% females *vs.* 6% males	Mattano *et al.* (abstr.)[38]	2014
ALL-BFM 2000 07/00-07/06	ALL • 1-18 y Multicenter	84/1,737	4.7% CI	• ***0.5% 1-<6 y vs. 1.3% 6-<10 y vs. 14.5% 10-<15 y vs. 22.7% 15-<18 y (S)*** • 1-<10 y: 0.8% DEX *vs.* 0.6% PRED; 10-<18 y: 13.8% DEX *vs.* 19.2% PRED (NS)	Möricke *et al.*[35]	2016
NOPHO ALL2008 06/09-ongoing	ALL • 1-17 y Multicenter	29/934	3.1%	• ***1.5% 1-9 y vs. 11.0% 10-14 y vs.*** 6.5% 15-17 y (S)	Toft *et al.*[36]	2016

PRED: prednisolone; DEX: dexamethasone; CI: cumulative incidence; S: significant, NS: not significant; y: years; Ind: Induction; Intensif: intensification; Maint: maintenance; Cont: continuation; Reind: reinduction; Postrem: postremission; Reintensif: reintensification; Cons: consolidation; ALL: acute lymphoblastic leukemia; ON: osteonecrosis; T-ALL: T cell acute lymphoblastic leukemia; WBC: white blood count; pts: patients; orthop. interv.: orthopedic interventions; MRI: magnetic resonance imaging; NCI: National Cancer Institute; NHR: non high-risk; HR: high-risk; EFS: event-free survival.

of dexamethasone and compared cumulative prednisone-equivalent doses of GCs, showing no significant correlation with the occurrence of osteonecrosis.[10,33,46] However, as dexamethasone is known to be more toxic to the skeletal system than prednisone, and low dexamethasone clearance was linked to severe osteonecrosis,[6] this approach might conceal differences. On the contrary, the toxic effects of dexamethasone during delayed intensification may be additive or synergistic with those of GCs administered during the induction phase. Reducing the duration of exposure to dexamethasone seems to reduce the risk for symptomatic osteonecrosis and outweighs the cumulative dose as a risk factor for the development of treatment-related osteonecrosis.[8,39,49]

GCs might affect antithrombin and protein S levels, with the latter further worsened by the additional administration of asparaginase, thus leading to hypercoagulability.[50]

Nonglucocorticoid drugs

Given the varying frequencies of osteonecrosis in different ALL treatment regimens, nonglucocorticoid drugs such as asparaginase (ASP) and methotrexate (MTX) may additionally modify the risk of osteonecrosis.[51] ASP treatment leads to increased plasma concentrations of dexamethasone,[6,51,52] whereas ASP allergy is associated with decreased systemic exposure to ASP and with decreased risk of osteonecrosis.[53] These effects might further be influenced by the different preparations of ASP used and, to some extent, explain the above-mentioned conflicting results regarding the risk of osteonecrosis in older patients, with the administration of dexamethasone compared to prednisone in trial ALL-BFM 2000 (native asparaginase)[35] and AALL0232 (pegylated asparaginase).[38]

High-dose MTX can damage the growth plate and primary bone, and the long-term use of MTX can reduce primary bone formation, likely due to decreased osteoblast function as well as increased osteoclast formation and function.[54,55] Methylenetetrahydrofolate reductase (MTHFR) polymorphisms can lead to mild to moderate increases in plasma homocysteine levels with homocysteinemia, leading to an increased risk of venous thrombosis.[56,57] Alkylating agents may harm gonadal function and lead to primary hypogonadism, which compromises bone mineralization if not adequately treated.[58] Ifosfamide can induce renal tubulopathy/Fanconi syndrome, and may subsequently manifest as hypophosphatemic rickets, compromising bone structure.[59] Due to hypercoagulability, vascular endothelial damage, and disruption of bone formation, purine antimetabolites can impair proliferation of chondrocytes.[60]

Other treatment related factors

Compared to chemotherapy alone, patients undergoing hematopoietic stem cell transplantation are at an increased risk of developing osteonecrosis.[3] Furthermore, total body irradiation (TBI) and chronic graft-*versus*-host disease correlate with the incidence of osteonecrosis.[61]

Non-treatment related factors

Osteonecrosis occurs more frequently in white patients than in black patients and in non-Hispanics than in Hispanics.[7,9,40] Girls between the ages of 10 and 14 years old are especially affected by osteonecrosis, whereas boys are at the highest risk above the age of 15 years.[7,46] There is no clear consensus on a risk difference between males and females. Even in groups that used essentially the same treatment regime, there are disparate results in this regard.[7,10,32,34,45,46,62] Inconsistent results have also been reported for the influence of obesity and BMI as risk factors.[8,63,64]

Table 4. Overview of MRI screening studies for osteonecrosis in children and adolescents with ALL.

Protocol & recruiting period	Study cohort No. of participating centers	No of patients (with ON/ALL)	Assessment	Incidence	Risk factors	Reference	Year
Total Therapy XIIIA, NHL XIII 12/91-08/94	ALL, advanced-stage NHL • <18 y Single center	17/116 incl. 6 asympt. 1 pt with orthop. interv.	Classified acc. to Ficat (earliest MRI 1 year after ALL diagnosis)	15.5%	• Age >10 y (S) • Sex, WBC, BMI, MTX dose, steroid dose, DEX (NS)	Ribeiro *et al.*[33]	2001
Nordic ALL protocols 07/92-12/05	ALL • 1-16 y 2 centers 3 pts with orthop. interv.	23/97 incl. 7 sympt.	At the end of therapy	24%	• 6% SR *vs.* 30% IR *vs.* 35% HR • High BMI, female sex, older age, higher cumulative DEX dose (S) • 7% ≤2 weeks *vs.* 36% >3 weeks DEX during delayed-intensification (S) • no difference in prednisone equivalents	Niinimäki *et al.*[8]	2007
St. Jude total XV 06/00-07	ALL Single center	69/364 exclud. 190 asympt.	after reind. I & II and at completion of therapy	71.8% CI any ON 17.6% CI sympt ON	• Age >10 y, SR/HR treatment arm (S) • Older age, lower albumin, higher lipid levels, poor DEX clearance (S)	Kawedia *et al.*[6]	2011

DEX: dexamethasone; S: significant; NS: not significant; y: years; Reind: reinduction; Pt: patient; incl: inclusive; ALL: acute lymphoblastic leukemia; ON: osteonecrosis; NHL: non Hodgkin lymphoma; MRI: magnetic resonance imaging; WBC: white blood count; BMI : body mass index; MTX: methotrexate; SR : standard-risk; IR: intermediate-risk; HR: high-risk; asympt: asymptomatic; orthop. interv.: orthopedic intervention(s);sympt : symptomatic; acc: according.

Genetic risk factors

Various genetic risk factors for the development of osteonecrosis in children with ALL and in steroid-induced osteonecrosis have been identified in numerous studies using candidate gene approaches and large genome-wide association studies (GWAS).[6,9,65-68]

Polymorphisms in the plasminogen activator inhibitor-1 (*PAI-1*) gene were initially reported to be associated with an increased risk of osteonecrosis,[4,66] but this finding could not be confirmed by subsequent GWAS studies.[68]

Table 5. Overview of cumulative corticosteroid doses in pediatric ALL (acute lymphoblastic leukemia) studies.

Protocol	Cumulative steroid dose (assigned to treatment phase)
Retrospective studies	
CCG-1882	*Ind.* (PRED, 28 d plus taper): 1,815 mg/m^2 *Regimens A+B*: *Delayed intensif.* (DEX, 21 d plus taper): 235 mg/m^2; *Maint.* (PRED, 5 d cycles): Males 7,000 mg/m^2, females 4,400 mg/m^2 *Regimen C: Delayed intensif. (*DEX, 21 d plus taper): 470 mg/m^2; *Maint.* (PRED, 5 d cycles): Males 6,200 mg/m^2, females 3,600 mg/m^2
DFCI 87-01 DFCI 91-01	*DFCI 87-01 Ind.* (PRED, 21 d): 840 mg/m^2; *Intensif./cont.* (PRED, 5 d cycles): SR 6,760 mg/m^2, HR 20,400 mg/m^2 *DFCI 91-01 Ind.* (PRED, 21 d): 1,120 mg/m^2; *Intensif./cont.* (DEX, 5 d cycles): SR 1,020 mg/m^2, HR 3,060 mg/m^2
AIEOP-ALL 95	*Ind.* (PRED, 28 d): 1,680 mg/m^2; *Reind.* (DEX, 21 d): 210 mg/m^2; *Cont.* (PRED, 5 d once): 200 mg/m^2 HR only
ALL-BFM 95	*Ind.* (PRED, 28 d plus taper): 1,837 mg/m^2, 1,417 mg/m^2 in HR only; *Reintensif.* (DEX, 22 d plus taper): 236 mg/m^2; *Int. interim cons.* (DEX, 5 d each cycle): 600 mg/m^2 MR only
UKALL97 *UKALL97/01*	PRED 7,728/11,019/7,117/9,938 mg/m^2; DEX 1,230/1,652/1,067/1,490 mg/m^2
CoALL-07-03	*LR red, LR stand, HR red, HR stand: Ind.* (PRED, 28 d): 1,680 mg/m^2; Reind. (DEX) *LR red* (7 d): 70 mg/m2; *LR stand* (14 d). 140 mg/m^2; *HR red* (2x7 d): 140 mg/m^2; *HR stand* (2x14 d): 280 mg/m^2
UKALL 2003	*Ind.* (DEX, 28 d): 168 mg/m^2 plus taper; *IM 1* (2x5 d): 60 mg/m^2; DI 1 (2x7 d): 140 mg/m^2; *IM 2* (2x5 d): 60 mg/m^2; *DI 2* (2x7 d): 140 mg/m^2; *Maint.* (3x5 d): 90 mg/m^2 per cycle, girls 7-8 cycles, boys 11-12 cycles
ANZCHOG 8	*Ind.* (PRED, 28 d plus taper); *Reind.* (DEX, 21 d plus taper) Cumulative steroid exposure 3,143 mg/m^2 prednisolone equivalents
DFCI 05-001	*Ind.* (PRED, 28 d): 1,120 mg/m^2 plus prophase; *Cons IC VHR only.* (DEX, 5 d): 90 mg/m^2; *CNS* (DEX, 5 d): SR 30 mg/m^2, HR/VHR 90 mg/m^2; *Cons II* (DEX, 5 d/cycle): SR 30 mg/m^2, HR/VHR 90 mg/m^2 approx. 9 cycles; *Cont.* (DEX, 5 d/cycle): 30 mg/m^2
Prospective studies	
ALL97 ALL97/99	*ALL97*: *Ind.* (28 d): PRED 1,120 mg/m^2 *vs.* DEX 182 mg/m^2; 1. *Intensif.* (PRED, 7 d): 280 mg/m^2; *CNS–dir. treat. (*PRED, 5 d every 4 weeks): 600 mg/m^2; 2. *Intensif.* (PRED, 7 d): 280 mg/m^2; *Interim CT* (PRED, 5 d every 4 weeks): 600 mg/m^2; 3. *Intensif.* (DEX, 10 d plus taper): 100 mg/m^2; CT (PRED, 5 d every 4 weeks): 3,200 mg/m^2 *ALL97/99:* Ind. (28 d plus taper): PRED 1,160 mg/m^2 *vs.* DEX 188,5 mg/m^2; *Interim maint. 1* (2x5 d): PRED 400 mg/m^2 *vs.* DEX 65 mg/m^2; *Delayed intensif. 1* (2x7 d): DEX 140 mg/m^2; *Interim maint. 2* (2x5 d): PRED 400 mg/m^2 *vs.* DEX 65 mg/m^2; *Delayed intensif. 2* (2x7 d): DEX 140 mg/m^2; Cont. (5x5 d): PRED 600 mg/m^2 *vs.* DEX 97,5 mg/m^2
DCOG-ALL9	*Ind.* (6 weeks) & repetitive pulses during maintenance; NHR 1,370 mg/m^2 DEX; HR 1,244 mg/m^2 DEX
CCG-1961	*Ind.* (PRED, 28 d plus taper): 1,815 mg/m^2; *Delayed intensif. A* (DEX, 21 d); *Delayed intensif. B* (DEX, 2x7 d)
DFCI-ALL 00-01	*Ind.* (PRED, 28 d): 1,120 mg/m^2 plus taper; *Intensif.* (10x5 d per cycle): DEX 300 *vs.* PRED 2,000 mg/m^2; *Cont.* (5 d per cycle): DEX 30 *vs.* PRED 200 mg/m^2 approx. 23 cycles
EORTC-CLG 58951	*Ind.* R1 (*28 d plus taper*): PRED 1,680 mg/m^2 *vs.* DEX 168 mg/m^2; *Reind.* (DEX, 21 d plus taper): 126 mg/m^2; *Maint.* (6x7 d): PRED 2,520 mg/m^2 *vs.* DEX 252 mg/m^2; VHR only: *Cons.* (DEX 3x5 d): 150 mg/m^2; *R-Blocks* (DEX 3x5 d): 150 mg/m^2
COG-AALL043	*Ind.* (PRED, 28 d): 1,680 mg/m^2; *Delayed intensif.* (DEX, 21 d): 1-9 y 210 mg/m^2; *Maint.* (DEX, 5 d): 30 mg/m^2 every 4 weeks *after 9/2008: Delayed intensif.* (DEX, 2x7 d): 140 mg/m^2; *Maint.* (PRED, 5 d): 200 mg/m^2 every 4 weeks; *Maint.* 1 year longer in males
ALL-BFM 2000	*Ind.* (28 d): DEX 280 mg/m^2 *vs.* PRED 1,680 mg/m^2 plus pre-phase and taper; *Reind./Prot. II* (DEX, 21 d plus taper): 210 mg/m^2; *Reind./Prot.* III (DEX, 14 d plus taper): 140 mg/m^2; *HR-Blocks* (DEX, 3x5 d): 300 mg/m^2
NOPHO ALL2008	*Ind.* preB-ALL and WBC <100x10^9/L (PRED, 28 d): 1,680 mg/m^2; T-ALL a/o WBC ≥100x10^9/L (DEX, 21 d): 210 mg/m^2; HR Block B1 (DEX. 5 d): 100 mg/m^2
MRI screening studies	
Total Therapy XIIIA, NHL XIII	*Ind.* (PRED, 28 d plus taper): 1,120 mg/m^2; *Cont. HR* (PRED, 7 d cycles): 280 mg/m^2 every 4 weeks; *Reind. HR* (PRED, 28 d plus taper): 1,120 mg/m^2; *Postrem. LR* (PRED, 7 d cycles): 280 mg/m^2 every 4 weeks
Nordic ALL protocols	*SR* 86 PRED 4,800 mg/m^2; *SR* 92 *PRED* 4,740 mg/m^2; *SR* 00 *PRED* 2,400 mg/m^2, DEX (5 d) 150/390 mg/m^2 *IR* 86 PRED 1,980 mg/m^2, *delayed intensif.* (28 d plus taper): DEX 320 mg/m^2; *IR* 92 PRED 4,260 mg/m^2, *delayed intensif.* (21 d plus taper) DEX 250 mg/m^2; *IR* 00 PRED 2,400 mg/m^2, *intensif.* (14 d) DEX 264/504 mg/m^2 *HR* 92 PRED 2,800/3,200 mg/m^2, *delayed intensif.* (21 d plus taper) DEX 240 mg/m^2; *HR 00* PRED 2,400 mg/m^2, intensif. (14 d) DEX 430 mg/m^2
St. Jude total XV	*Cont.* (DEX, 3x5 d): LR 120 mg/m^2, SR/HR 180 mg/m^2 *Reind. I & II* (DEX, 2x4x8 d): 448 mg/m^2

PRED: prednisolone, DEX: dexamethasone; Ind: Induction; Intensif : intensification; Maint: maintenance; Cont: continuation; Reind: reinduction; Postrem: postremission; Reintensif: reintensification; Cons: consolidation. SR: standard-risk; HR: high-risk; MR: medium-risk; LR: low-risk; VHR: very high-risk; IC VHR: consolidation 1C very high-risk; NHR: non high-risk; CT: continuing therapy; CNS : central nervous system; d: day(s); preB-ALL: precursor B acute lymphoblastic leukemia; WBC: white blood count; T-ALL: T cell acute lymphoblastic leukemia; IR: intermediate-risk.

Likewise, findings about polymorphisms involved in lipid homeostasis (acid phosphatase locus 1, *ACP1*),[6] antifolate pharmacodynamics (thymidylate synthetase, *TYMS*), and steroid hormone response (vitamin D receptor, *VDR*), have been reported to be associated with osteonecrosis,[9] but were not reproducible in GWAS studies.[68]

According to recent GWAS studies, the glutamate receptor pathway seems to be of crucial importance for the pathogenesis of osteonecrosis in patients with prolonged exposure to corticosteroids. Mechanical load opens mechanosensitive calcium channels in osteocytes, leading to exocytosis of glutamate, which activates osteoblast receptors and impairs endothelial barrier function.[67-70] In addition, SNPs in adipogenesis pathways and in enhancers active in mesenchymal stem cells are significantly associated with osteonecrosis development.[67] Bone morphogenetic protein (*BMP*) is toxic to vascular smooth muscle and is released in response to bone damage and mechanical stress.

To summarize, osteonecrosis risk is influenced by germline polymorphisms in genes linked to pharmacodynamics of chemotherapy, bone metabolism, adipogenesis, glutamate signaling pathway, and mesenchymal stem cell differentiation. However, given the lack of a single consistent genetic factor being undoubtedly identified, predictive diagnostic testing that helps to evaluate the risk of osteonecrosis development is not established. Even in the context of genetic variants that increase the risk of osteonecrosis, the occurrence of osteonecrosis remains highly dependent on the patient's age and the specific therapeutic regimen, and, conversely, genetic risk factors significantly depend on the patient's age.

Adolescence

Age is the most consistently identified and most significant risk factor, with patients ≥10 years old at the highest risk across treatment regimens and study groups (Table 1).[7-11,43,62] In contrast, the incidence is lower in adults undergoing ALL therapy.[36] Thus, the pathogenesis of osteonecrosis is likely strongly associated with factors being most prominent in adolescent age, thereby causing the highest vulnerability for osteonecrosis in this age.

There are several adolescent physiological processes that differ fundamentally from younger children and older individuals. These can mainly be attributed to hormonal changes that might lead to increased osteonecrosis susceptibility *via* interaction with different mechanisms, such as increased local metabolic/perfusion requirements, skeletal maturation (e.g., growth plate structure and development), the coagulation system, or osseous blood vessel supply.

All these processes are induced by the beginning and maturation of sexual hormone production and a physiological peak of growth hormone production during puberty.

Increasing sexual hormone and especially estrogen concentrations during puberty have procoagulatory effects, thus adolescence is associated with the highest risk of the development of venous thromboembolism.[71] Additional risk factors, such as thrombophilia or hypofibrinolysis, can further increase the risk of thrombosis.[25,72] In experimental settings, testosterone increases nitrogen monoxide release of endothelial cells, and inhibits platelet aggregation.[73] Estrogen further promotes intracortical bone remodeling. Bone material is added to the endosteal surface, increasing cortical density and bone mass during puberty.[22] These estrogenic effects result in a peak in bone mass gain, and the procoagulatory effects of estrogens predispose adolescents to an imbalance between osseous metabolic/blood supply demands and actual osseous blood supply. This effect might further explain the trend to witness more osteonecrosis in females compared to males.

The growth hormone/IGF1 axis is physiologically stimulated during puberty, 1.5- to 3-fold compared to pre- and postpubertal individuals.[74] The IGF1 level peaks in females at an average of 14 years, and in males at 15 years of age.[75] Peak growth velocity/pubertal growth spurt can be expected at 12 years of age in females, and at 14 years of age in males.[76] This leads to excessive metabolic activity in growth plates and bones, such as increased oxygen consumption with increased hypoxic effects in growth plates.[77-79] This helps to explain why areas of bone with late epiphyseal closure and extensive contribution to pubertal length growth, such as long limb bones, are predominantly affected by osteonecrosis.[7]

Pubertal epiphyseal maturation and ossification progressively reduce mechanically compliant areas in bone architecture, which might then lose their ability to compensate for increased bone marrow pressure.

Concentrations of pro- and anticoagulant factors change crucially during growth.[80,81] Major turning points occur after the first six months of life, and between adolescence (11–16 years) and adulthood.[82] Coagulant factors (II, V, VII, IX, X, XI, XII, bleeding time), anticoagulant factors (α2M, HCII, Protein C), and the fibrinolytic system (plasminogen, TPA, PAI) are substantially modulated during adolescence and differ significantly from adult levels.[80] Both elevated estrogen and testosterone levels further increase the impact of underlying thrombophilia (Factor V Leiden, MTHFR polymorphisms, prothrombinemia, Protein C deficiency, Protein S deficiency hyperhomocysteinemia), and hypofibrinolysis (PAI polymorphisms, increased plasminogen activator inhibitor activity).[4,25,66,83] In addition, lifestyle factors, such as smoking, substance abuse, obesity, and use of oral hormonal contraceptives gain importance during adolescence and further contribute to venous thromboembolism (VTE) risk.[71] For example, contraceptives with high estrogen content influence the protein C pathway, with subsequently increased activated protein C (APC) resistance[84] and platelet aggregation.[85] This likely increases the risk of intraluminal obliteration and ischemia in the rapidly growing bone.

Prevention and screening

Long continuous exposure during delayed intensification plays a pivotal role in the development of osteonecrosis. Therefore, this was modified in two trials, either by replacing continuous with alternate-week dexamethasone or by entirely reducing the duration of administration.[8,39] osteonecrosis incidence in patients treated according to the altered dexamethasone schedule was significantly reduced. However, high-risk ALL patients with osteonecrosis had a 17.6% better event-free survival than patients without osteonecrosis.[39] Hence, modifying treatment must be carefully monitored in future prospective trials.

A different approach to reduce osteonecrosis-associated debilitating long-term effects is early screening for osteonecrosis by MRI to prevent functional impairment. This has been carried out by Ribeiro *et al.*[33] one year after diagnosis of ALL, by Niinimäki *et al.*[86] at the end of therapy, by Kaste *et al.*[87] at 6.5 and 9 months from diagnosis and at completion of therapy, and by Kawedia *et al.*[6] after reinduction I and II and at completion of therapy. The number of patients diagnosed with radiographic osteonecrosis was high (15.5%,[33] 24%[86] and 71.8%,[6] respectively) with a substantial proportion of patients remaining asymptomatic until the end of the study (35%, 70%, and 73%, respectively). Kaste *et al.*[87] further distinguished between limited and extensive (involving more than 30% of the head surface) femoral head osteonecrosis, the latter being a crucial predictor of joint infraction. As radiological classification of osteonecrosis was not uniform, comparability of these results is limited. Furthermore, a recent study by Niinimäki *et al.* identified critical deficiencies in all available radiological osteonecrosis classification systems and recommended a new, joint-specific classification system.[88]

With a cumulative incidence of 71.8% of any osteonecrosis, the study by Kawedia *et al.*[6] highlights the need for further research, with particular regard to follow-up, as the course of osteonecrosis may be transient and reversible and some changes may resolve without symptoms. Even when patients present with joint pain and radiographic changes, the clinical course remains unpredictable. Thus, identifying patients at risk of functional impairment and debilitating progressive joint disease still remains challenging.

Precise prospective evaluation of side effects and toxicity in children undergoing treatment for ALL in childhood is therefore an important aspect of modern therapy to reduce compromising outcome after successful treatment. Hence, we initiated the multi-center OPAL trial (**O**steonecrosis in **P**ediatric patients with **A**cute lymphoblastic **L**eukemia and lymphoblastic lymphoma [LBL]), which is still ongoing. In this trial, we prospectively evaluate children aged ≥10 years diagnosed with ALL or LBL, who are treated according to the AIEOP-BFM 2009 and the CoALL-08-09 protocol with a combination of MRI screening and symptom-oriented anamnesis and functional examination at defined time points during ALL treatment. The trial aims to define the proportion of children who can be diagnosed with early asymptomatic osteonecrosis by MRI and subsequently develop symptomatic osteonecrosis, to identify critical time points of osteonecrosis development during ALL treatment, and to describe the natural course of asymptomatic osteonecrosis lesions only identified by MRI. These data are still lacking and are mandatory for the subsequent evaluation of interventions aimed at preventing osteonecrosis progression and functional impairment. These aspects strongly underline the need for intensive future research in the field of pediatric osteonecrosis.

Conclusions

Osteonecrosis is the most common therapy-related side effect in children with acute lymphoblastic leukemia. Better understanding of the associated therapy-related and non-therapy-related risk factors is needed to improve prediction, management, and, preferably, prevention of this sequelae.

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