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ORIGINAL ARTICLE

# Genetic polymorphism of vitamin D receptors and plasminogen activator inhibitor-1 and osteonecrosis risk in childhood acute lymphoblastic leukemia

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**Abstract**

**Background:** Osteonecrosis (ON) is one of the major therapy-related complications in childhood acute lymphoblastic leukemia (ALL). The purpose of the current study is to assess the frequency of ON in children with ALL and to detect whether polymorphisms in vitamin D receptor gene (VDR) and plasminogen activator inhibitor-1 (PAI-1) gene can affect the risk of ON.

**Patients and Methods:** Nighty-six ALL children were enrolled. Serum 25-hydroxyvitamin D 25(OH)D levels were performed in addition to the detection of polymorphisms in PAI-1and VDR genes by polymerase chain reaction.

**Results:** Ten out of 96 patients had ON (four males and six females aged above 10 years) and had an insufficient level of 25(OH)D. Fifty-two percent of patients had PAI-1 GG genotype while 48% had PAI-1 GA genotype. PAI-1 polymorphism was detected in 60% of all ON cases. The frequencies of VDR genotypes were CT (56.3%), CC (39.6%), and TT (4.2%). Osteonecrosis was found in eight patients with CC genotype and in two patients with CT genotype.

**Conclusion:** Osteonecrosis can develop early during the therapy of ALL. Older age and insufficient level of 25(OH)D were considered important risk factor for the development of osteonecrosis. PAT-1 and VDR gene polymorphism may be a genetic risk factor in its pathogenesis.

**KEYWORDS**

ALL, osteonecrosis, PAI-1, pediatric, polymorphism, VDR

## 1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in children, representing one-quarter of all pediatric cancers (Seibel et al., 2008). The survival of children with acute lymphoblastic leukemia has increased in the last years due to the progressive intensification of multiagent chemotherapy. 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 (Karol et al., 2015). Multiple candidate gene studies have indicated several polymorphisms in genes related to the development of osteonecrosis, such as PAI-1 gene (French et al., 2008) and VDR gene (Relling et al., 2004).

Polymorphisms in PAI-1 gene and VDR genetic variants have been associated with bone and vasculature morbidity, these pharmacogenetic associations likely reflect the interaction of antileukemic medications with germline sensitivity to drug action and might identify ALL patients at highest risk to develop osteonecrosis (Relling et al., 2004).

However, there was a debate about the association of plasminogen activator inhibitor-1 gene and osteonecrosis in acute lymphoblastic leukemia (Bond et al., 2011).

Therefore, we aimed to study the frequency of ON in children with ALL and to detect whether polymorphisms in VDR and PAI-1 gene may affect the risk of osteonecrosis in childhood ALL.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients

This prospective study was carried on 96 children with acute lymphoblastic leukemia who were diagnosed and treated at the Pediatric Oncology Unit, Zagazig University Hospitals, during the period from September 2018 to June 2020. Their age ranges from 3 to 15 years, they were 62 males and 34 females. The study was approved by the Institutional Review Board, Faculty of Medicine, Zagazig University. Informed consent and ascent forms were obtained from the study participants and/or their guardians.

All patients were subjected to full medical history, thorough clinical examination with specific attention to the musculoskeletal system, particularly bone pain and loss of function in addition to anthropometric measures such as weight, height, and body mass index.

All enrolled patients on the current work were newly diagnosed ALL cases who were treated according to ALL Protocol adopted from St. Jude total XV protocol. They underwent induction therapy with prednisolone oral tablets 40 mg/m$^2$ daily dose for 28 days, L-asparaginase 10.000 IU/m$^2$/dose IM thrice weekly, vincristine 1.5/m$^2$ weekly for four doses, doxorubicin 25 mg/m$^2$ for two doses, cyclophosphamide 1000/m$^2$, 6-mercaptopurine 60 mg/m$^2$ for last 2 weeks, cytarabine 75 mg/m$^2$ for eight doses and triple intrathecal therapy. At the end of induction, the patients were stratified to low, standard, or high-risk arms according to their risk of ALL relapse (Pui et al., 2009). Patients assigned as high/standard risk received consolidation therapy of high dose methotrexate (MTX) 5 gm/m$^2$ every other week for four doses, daily 6-mercaptopurine 50 mg/m$^2$ in addition to triple intrathecal therapy, while low-risk patients received lower dose of MTX 2.5 gm/m$^2$.

### 2.2 | Methods

1. Laboratory investigations: 5 ml of venous blood were aseptically withdrawn from each patient for estimation of serum 25(OH)D levels at diagnosis of acute lymphoblastic leukemia and after receiving consolidation therapy in addition to the detection of PAI-1 gene and VDR gene polymorphism by polymerase chain reaction at diagnosis.
   I. Serum 25(OH)D levels were measured by an ELISA kit supplied by DRG International, Inc. USA with Catalog Number (EIA-5396), according to the manufacturer protocol. Serum 25(OH)D levels >30 ng/ml are considered as sufficient; 20–30 ng/ml are insufficient while <20 ng/ml are deficient (Holick, 2011).
   II. Testing of PAI-1and VDR genes polymorphism:
      a. Genomic DNA extraction using a spin column method.
      b. Amplification of Plasminogen activator inhibitor-1 gene (PAI-1) and Vitamin D receptor gene (VDR) by PCR.
      c. Agarose gel electrophoresis using Submarine Gel electrophoresis system and Submarine chamber and photographed under ultraviolet light.
      d. Genotyping of PAI-1 rs6092 (G>A) using (REFLP-PCR). The amplified PCR product was then digested with FokI restriction endonuclease (Fermentas, Germany). Digested product was electrophoresed on 2% agarose gel. The VDR wild-type CC gives 265 bp whereas the mutant TT gives 196 and 69 bp. Heterogenous CT genotype gives 265, 196, and 69 bp.
2. Imaging: Magnetic resonance imaging (MRI) on hip and knee was performed for all patients at diagnosis and after the consolidation phase of therapy.

MRI imaging of knees and hips was performed using Siemens 1.5 Tesla magnet (Siemens, Erlanen, Germany). In this study, ON was defined as a geographic lesion with a distinct rim of low signal intensity in the normally high-intensity marrow on T1-weighted images (the band sign) and high signal intensity in the normally low-intensity marrow on short tau inversion recovery (STIR) images (double-line sign) (Ojala et al., 1999). Osteonecrosis at the time of each MRI was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0, and osteonecrosis was categorized as absent (grade 0), asymptomatic (grade 1), or moderate (grade 2), severe (grade 3), or disabling (grade 4).

MRI studies were reviewed by a single pediatric radiologist experienced in pediatric oncologic and orthopedic imaging

## 3 | STATISTICAL ANALYSIS

The data were analyzed using SPSS version 20 (IBM Corp.). Continuous Quantitative variables were expressed as the mean ± SD & median (range). Comparison of several groups' median was performed by Kruskal–Wallis and comparison between two groups was performed by Mann–Whitney *U* test. Categorical qualitative variables were expressed as absolute frequencies & relative (number & percentage) and the comparison of several groups frequencies was performed by chi-square test ($X^2$). The results were considered statistically significant when *p* value is ≤0.05.

## 4 | RESULTS

Nighty-six ALL patients were recruited in the current study, their mean age was 8.08 ± 3.79 years, ranged from 3 to 15 years, 65% of them were males, and 35% were females. The mean body mass index (BMI) was 16.01 ± 0.96 kg/m$^2$, with a range from 14.5 to 18.3 kg/m$^2$.

Forty-seven patients were low risk, 37 patients were standard risk and 10 patients were high risk according to Pui et al., (2009) risk classification. Genotyping was performed for PAI-1 and VDR for ALL patients at diagnosis. PAI-1 GG genotype was found in 52% and PAI-1 GA genotype was found in 48% of patients, while VDR CT genotype was found in 52%, CC genotype was found in 40% and only 8% expressed TT genotype (Table 1).

The distribution of 25(OH)D levels is presented in (Figure 1). Twenty-two patients (22.9%) were vitamin D deficient (<20 ng/dl) and 48 (50%) were vitamin D insufficient (20–30 ng/ml) at diagnosis, while 10 (10.4%) patients were deficient and 58 (60.4%) patients were insufficient after consolidation. At diagnosis, only 26 (27%) patients were sufficient (>30 ng/dl), while 58 (60.4%) patients were sufficients after consolidation.

MRI evaluations of both hip and Knee joints were performed at diagnosis and after consolidation for all patients. Ten patients developed ON (overall incidence 10.4%). Two patients developed grade III ON of both knee joints, while the other eight patients had grade 2–4 ON of both hip joints after consolidation. All of the 10 patients with ON were among the standard and high risk groups.

The clinical and laboratory characteristics of patients with and without ON are listed in Table 2. Ten (10.4%) out of 96 patients developed osteonecrosis, six females and four males. Their age was above 10 years old. All patients who developed osteonecrosis had insufficient vitamin D level at diagnosis and after consolidation therapy. Eight patients (80%) who developed osteonecrosis had VDR CC genotype, while six patients had PAI-1 GA genotype.

**TABLE 1** Clinical data and gene polymorphism among studied acute lymphoblastic leukemia patients

| | Studied patients (*N* = 96) | |
|---|---|---|
| | No. | % |
| Demographic data | | |
| Sex | | |
| Male | 62 | 65.0 |
| Female | 34 | 35.0 |
| Age (years) | | |
| Mean ± SD | 8.08 ± 3.79 | |
| Median (range) | 9(3–15) | |
| Anthropometric parameters | | |
| Weight (kg) | | |
| Mean ± SD | 24.2 ± 8.98 | |
| Median (range) | 27(13–40) | |
| Height (m) | | |
| Mean ± SD | 120.8 ± 22.24 | |
| Median (Range) | 130(90–152) | |
| BMI (kg/m$^2$) | | |
| Mean ± SD | 16.01 ± 0.96 | |
| Median (range) | 16(14.5–18.3) | |
| Gene polymorphism | | |
| PAI-1 | | |
| GA | 46 | 48.0 |
| GG | 50 | 52.0 |
| VDR | | |
| CT | 50 | 52.0 |
| TT | 8 | 8.0 |
| CC | 38 | 40.0 |

The mean serum 25(OH)D level at diagnosis was 27.46 ± 7.89 ng/ml in patients with PAI-1GG genotype versus 22.7 ± 4.81 ng/ml in patients with PAI-1 GA genotype with statistically significant difference ($p < .005$). While, at the end of consolidation therapy, the mean serum vitamin D level was 39.5 ± 5.85 ng/ml in patients with PAI-1 GG genotype versus 27.85 ± 10.44 ng/ml in patients with PAI-1 GA genotype with statistically significant difference ($p < 0.05$) (Table 3).

The mean serum vitamin D level at diagnosis in patients with VDR gene polymorphism was 24.12 ± 7.8, 23.5 ± 3.1, and 26.82 ± 5.7 ng/ml in VDR CT, VDR TT, and VDR CC genotypes respectively with no statistically significant difference ($p > 0.05$) (Table 4).

The mean serum vitamin D level at the end of consolidation was 34.56 ± 10.47, 34.28 ± 2.8, and 21.7 ± 3.3 ng/ml in VDR CT, VDR CC, and VDR TT genotypes, respectively,

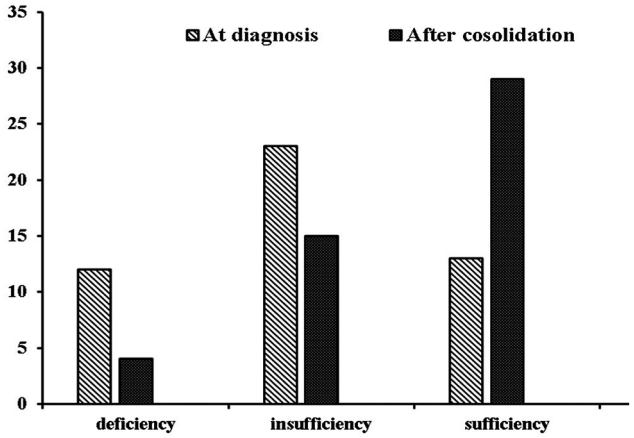


**FIGURE 1** The distribution of 25-hydroxyvitamin D levels among acute lymphoblastic leukemia patients at diagnosis and after consolidation therapy

with no statistically significant difference $p > 0.05$. No significant relationship was found between vitamin D status and any VDR polymorphism genotypes at diagnosis and after consolidation therapy ($p > 0.05$) (Table 4).

## 5 | DISCUSSION

Cure rates for childhood acute lymphoblastic leukemia (ALL) have approached 90% with therapeutic advances over the last several decades (Schrappe et al., 2013).

However, one of the most common therapy-related and dose-limiting toxicities of therapy in children with ALL is glucocorticoid-induced osteonecrosis, particularly in those older than 10 years of age (Hunger et al., 2012).

In the present work, ON was reported in 10 patients (10.4%). The reported incidence of ON in literature is variable. Padhye et al., (2016) and Sakamoto et al., (2018) reported that the incidence of ON is 7% among their study population. Moreover, Mattano et al., (2000), Plesa et al., (2019), and Pui et al., (2009), reported that the prevalence of ON among their patients was 9.3%, 10.5%, and 12%, respectively. The published prevalence of ON in children with ALL varies widely depending on the definition of ON, treatment protocol, and selection of patients. The true prevalence could be underestimated as most published studies are retrospective (Barr & Sala, 2008; Elmantaser et al., 2010).

In the current study, we found a significant association between the age of patients and development of osteonecrosis as all children who developed osteonecrosis were older than 10 years, which may suggest older age as a risk factor for osteonecrosis.

Several studies also reported the age of the patients as an independent risk factor for ON (Karol et al., 2015; Kawedia et al., 2011; Mattano et al., ,2000, 2012; Rhodes et al., 2017; Te Winkel et al., 2011).

In fact, the increase of ON with older children has been suggested to result from higher circulating sex hormones levels and closure of the growth plates resulting in less buffering capacity of increased intraosseous pressure (Mattano et al., 2000).

Another risk factor for ON is sex. Many studies reported higher risk of ON in girls than boys (Aricò et al., 2003; French et al., 2008; Högler et al., 2007; Plesa et al., 2019; Strauss et al., 2001; Te Winkel et al., 2011), which was found in our study although other studies have not confirmed this (Barr & Sala, 2008; Mory et al., 2009).

High BMI theoretically may increase the risk of ON by reduced blood flow in bone marrow resulting from hypertrophic fat cells, by fat embolism, or by an increase of mechanical force in joints. Two small studies have described conflicting results regarding the role of BMI in developing AVN (Mory et al., 2009; Niinimaki et al., 2007). In our work, we found a significant association between BMI and development of osteonecrosis.

While corticosteroids are the main culprit for skeletal toxicity on childhood ALL. Other chemotherapeutic agents such as methotrexate and asparaginase additionally contribute to ON and reduced bone mineralization. These agents impair osteoblast function responsiveness and number which subsequently account for the suppression of bone formation and contribute to skeletal abnormalities noted during and after the treatment (Te Winkel et al., 2014).

Biologically and genetic basis for ON has been frequently investigated (Bond et al., 2011; Karol et al., 2015). PAI-1 inhibits fibrinolysis. Increased serum levels have been associated with increased risk of thrombophilia (Seguí et al., 2000) and osteonecrosis, although reports are not consistent (Asano et al., 2004; Kim et al., 2011). High levels of PAI-1, induced by corticosteroid treatment, or polymorphisms in PAI-1, lead to the suppression of fibrinolysis and promotion of thrombosis (Zhang et al., 2013), resulting in elevated intraosseous venous pressure and hence blocking blood flow to the femoral head which may end in hypoxic bone death or osteonecrosis (Lee et al., 2012; Seguí et al., 2000). A finding which was not present in any of our patients.

The frequency of PAI-1 gene polymorphisms among our patients was 52% for GG genotype and 48% for GA genotype while none had AA genotype. This is consistent with Bond et al., (2011), who found PAI-1 GG genotype in 77% and PAI-1 GA genotype in 19%. Also, French et al., (2008), reported PAI-1 GG genotype in 73% and PAI-1 GA genotype in 26%.

VDR polymorphism was known to be associated with osteopenia in non-cancer settings. Furthermore, VDR might play a role in the excretion of glucocorticoids (Whitfield

**TABLE 2** Clinical, bone mineral status, and genotyping in patients with osteonecrosis versus patients without osteonecrosis

| | Patients with osteonecrosis (10) | Patients without osteonecrosis (86) | test of significance | *p* |
|---|---|---|---|---|
| Sex | | | | |
| Male | 4 | 58 | 1.47[a] | 0.22 |
| Female | 6 | 28 | | |
| Age (years) | | | | |
| Mean ± SD | 12.8 ± 2.05 | 7.418 ± 3.56 | 22.500[b] | 0.002* |
| ≤10 years | 0 | 66 | 12.279[a] | 0.002* |
| ˃10 years | 10 | 20 | | |
| BMI | | | | |
| Mean ± SD | 17.14 ± 0.65 | 16.027 ± 0.96 | 32.500[b] | 0.008* |
| VITAMIN D status after consolidation | | | | |
| Deficiency (0–20) | 0 | 8 | 12.28[a] | 0.002* |
| Insufficiency (20–30) | 10 | 20 | | |
| Sufficiency (30–50) | 0 | 58 | | |
| VITAMIN D status initial | | | | |
| Deficiency (0–20) | 0 | 24 | 6.067[a] | 0.048* |
| Insufficiency (20–30) | 10 | 36 | | |
| Sufficiency (30–50) | 0 | 26 | | |
| PAI | | | | |
| GG | 4 | 42 | 0.708[a] | 0.14 |
| GA | 6 | 44 | | |
| VDR | | | | |
| CC | 8 | 30 | 3.8[a] | 0.05 |
| CT–TT | 2 | 56 | | |

*Statistically significant.

[a] Chi-Square test.

[b] Mann–Whitney to student t test.

et al., 2001). The truncated protein from the C-allele was linked to a higher affinity for 1,25-dihydroxyvitamin D (Eisman, 2001). VDR gene polymorphisms in our study were 52% of patients had CT genotype, 40% had CC genotype, and 8% had TT genotype. This is in agreement with French et al. (2008), who reported that CT/TT was found in 64% and CC was found in 35% of their study population. In contrast, Relling et al., (2004), reported that the frequency of VDR gene polymorphism was as follows; 48%, 39%, and 12% in CC, CT, and genotypes, respectively.

In the present study, 10 patients developed osteonecrosis, 4 with PAI-1 GG genotype, and 6 with PAI-1 GA genotype with no significant difference, which may be attributed to small sample size. French et al., (2008), studied different genetic polymorphisms in childhood ALL who developed osteonecrosis. They reported that only the PAI-1 polymorphism was associated with osteonecrosis. Among them, 26.9% had combined GA and AA genotypes and 11.7% had GG genotype.

In contrast, we found a significant association of VDR gene polymorphism with ON, as 80% of patients who developed osteonecrosis were CC genotype versus 20% in patients with CT genotype. Similarly, Relling et al., (2004), reported in their study that The VDR, CC genotype was more common in patients who developed osteonecrosis (51%), while the CT genotype was found in 33% of patients.

It is possible that other genetic modifiers could obscure any putative effect of the PAI-1 polymorphism. Monogenic prediction of the disease is not straightforward, particularly in the context of a disorder such as ON, which has multifactorial

**TABLE 3** Relation between PAI-1 genotypes and vitamin D in studied ALL patients at diagnosis and after consolidation therapy

| | PAI-1 | | | | | |
|---|---|---|---|---|---|---|
| | GA (N=46) | | GG (N=50) | | | |
| | No. | % | No. | % | Test | *p*-value |
| At diagnosis | | | | | | |
| Vitamin D level (ng/ml) | | | | | | |
| Mean ± SD | 22.7±4.81 | | 27.46±7.89 | | 39.500[a] | 0.035* |
| Vitamin D status | | | | | | |
| Deficiency | 8 | 17 | 16 | 32 | 1.105[b] | 0.576 |
| Insufficiency | 26 | 57 | 18 | 36 | | |
| Sufficiency | 12 | 26 | 16 | 32 | | |
| After consolidation therapy | | | | | | |
| Vitamin D level (ng/ml) | | | | | | |
| Mean ± SD | 27.85±10.44 | | 39.5±5.85 | | 30.000[a] | 0.008* |
| Vitamin D status | | | | | | |
| Deficiency | 8 | 17.4 | 0 | 0.0 | 9.547[b] | 0.008* |
| Insufficiency | 24 | 52.2 | 4 | 8 | | |
| Sufficiency | 14 | 30.4 | 46 | 92 | | |

*Statistically significant.

[a] Mann–Whitney *U* test.

[b] Chi-square test.

**TABLE 4** Relation between VDR genotypes and vitamin D in studied ALL patients at diagnosis and after consolidation therapy

| | VDR gene polymorphism | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CT (N=50) | | TT (N=8) | | CC (N=38) | | | |
| | No. | % | No. | % | No. | % | TestTest | *p*-value |
| At diagnosis | | | | | | | | |
| Vitamin D level (ng/ml) | | | | | | | | |
| Mean ± SD | 24.12±7.8 | | 23.5 ±3.1 | | 26.82±5.7 | | 0.923[b] | 0.63 |
| Vitamin D status | | | | | | | | |
| Deficiency | 18 | 36 | 0 | 0.0 | 4 | 10.5 | 3.457[a] | 0.485 |
| Insufficiency | 18 | 36 | 8 | 100.0 | 22 | 58 | | |
| Sufficiency | 14 | 28 | 0 | 0.0 | 12 | 31.5 | | |
| After consolidation therapy | | | | | | | | |
| Vitamin D level (ng/ml) | | | | | | | | |
| Mean ± SD | 34.56±10.47 | | 21.7 ±3.3 | | 34.28±2.834.28±2.8 | | 1.941[b] | 0.379 |
| Vitamin D status | | | | | | | | |
| Deficiency | 8 | 16 | 2 | 25 | 0 | 0.0 | 5.663[a] | 0.226 |
| Insufficiency | 8 | 16 | 4 | 50 | 16 | 42.1 | | |
| Sufficiency | 34 | 68 | 2 | 25 | 22 | 57.9 | | |

[a]Chi-square test.

[b]Kruskal–Wallis Test.

pathogenesis that remains poorly understood. Further work is necessary to characterize the genetic basis of therapy-related morbidity in ALL. Better tools of prediction would allow prospective identification of patients in whom timely treatment modification might reduce the risk of serious pathology (Kawedia et al., 2011).

Last, one important finding in the current study which was consistent with previous literature (Jackmann et al., 2020) was the high prevalence of vitamin D insufficiency and deficiency in the included ALL patients. We agree with other authors that vitamin D supplementation should be implemented in all patient with ALL all through their disease

course and to be continued even after remission(Delvin et al., 2019; Ladas et al., 2016; Reisi et al., 2015).

## 6 | CONCLUSION

From the present study, we concluded that osteonecrosis can develop early during therapy of ALL. Age older than 10 years and insufficient Vitamin D were detected as important risk factors. Inherited variation in PAT-1 and VDR may contribute to the risk of ON. Large-scale studies are still needed to support our findings.

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### CONFLICT OF INTEREST

All authors declare no competing interests related to the study.

### AUTHOR CONTRIBUTIONS

L.S., M.B., N.R., and M.Z.: set the idea of the study and designed the study. LS, MB, MZ, N.K: reviewed literature, drafted the manuscript, and critically analyzed the data. E.A., W.M., and H.E. recruited pediatric patients and collected their data. K.E, B.S: performed the radiological part of the study. N.R, H.S, G.M: performed the laboratory aspect of the study participants and the genetic mutation analysis. All authors reviewed and approved the manuscript for final publication.

### CONSENT FOR PUBLICATION

All parents of enrolled children signed written informed consents for publication in the current study.

### ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the research and ethical committee of the participating hospitals. All parents of enrolled children signed written informed consents for the participation of their children in the current study.

### DATA AVAILABILITY STATEMENT

All data and materials related to the study are included in the current manuscript.

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### REFERENCES

Aricò, M., Boccalatte, M. F., Silvestri, D., Barisone, E., Messina, C., Chiesa, R., Santoro, N., Tamaro, P., Lippi, A., Gallisai, D., & Basso, G. (2003). Osteonecrosis: An emerging complication of intensive chemotherapy for childhood acute lymphoblastic leukemia. *Hematologiaca*, *88*, 747–753.PMID: 12857552.

Asano, T., Takahashi, K. A., Fujioka, M., Inoue, S., Ueshima, K., Hirata, T., Okamoto, M., Satomi, Y., Nishino, H., Tanaka, T., Hirota, Y., & Kubo, T. (2004). Relationship between postrenal transplant osteonecrosis of the femoral head and gene polymorphisms related to the coagulation and fibrinolytic systems in Japanese subjects. *Transplantation*, *77*, 220–225. https://doi.org/10.1097/01.TP.0000101433.99651.96

Barr, R. D., & Sala, A. (2008). Osteonecrosis in children and adolescents with cancer. *Pediatric Blood & Cancer*, *50*, 483–485. https://doi.org/10.1002/pbc.21405

Bond, J., Adams, S., Richards, S., Vora, A., Mitchell, C., & Goulden, N. (2011). Polymorphism in the PAI-1 (SERPINE1) gene and the risk of osteonecrosis in children with acute lymphoblastic leukemia. *Blood*, *118*, 2632–2633. https://doi.org/10.1182/blood-2011-05-355206

Delvin, E., Alos, N., Rauch, F., Marcil, V., Morel, S., Boisvert, M., Lecours, M. A., Laverdière, C., Sinnett, D., Krajinovic, M., Dubois, J., Drouin, S., Lefebvre, G., Samoilenko, M., Nyalendo, C., Cavalier, E., & Levy, E. (2019). Vitamin D nutritional status and bone turnover markers in childhood acute lymphoblastic leukemia survivors: A PETALE study. *Clinical Nutrition*, *38*(2), 912–919. https://doi.org/10.1016/j.clnu.2018.02.006. Epub 2018 Feb 21 PMID: 29503055.

Eisman, J. A. (2001). Pharmacogenetics of the vitamin D receptor and osteoporosis. *Drug Metabolism and Disposition*, *29*, 505–512.

Elmantaser, M., Stewart, G., Young, D., Duncan, R., Gibson, B., & Ahmed, S. F. (2010). Skeletal morbidity in children receiving chemotherapy for acute lymphoblastic leukemia. *Archives of Disease in Childhood*, *95*, 805–809. https://doi.org/10.1136/adc.2009.172528

French, D., Hamilton, L. H., Mattano, L. A., Sather, H. N., Devidas, M., Nachman, J. B., & Relling, M. V. (2008). A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: A report from the Children's Oncology Group. *Blood*, *111*, 4496–4499. https://doi.org/10.1182/blood-2007-11-123885

Högler, W., Wehl, G., van Staa, T., Meister, B., Klein-Franke, A., & Kropshofer, G. (2007). Incidence of skeletal complications during treatment of childhood acute lymphoblastic leukemia: Comparison of fracture risk with the General Practice Research Database. *Pediatric Blood & Cancer*, *48*, 21–27. https://doi.org/10.1002/pbc.20701

Holick, M. F. (2011). Vitamin D: Evolutionary, physiological and health perspectives. *Current Drug Targets*, *12*, 4–18. https://doi.org/10.2174/138945011793591635

Hunger, S. P., Lu, X., Devidas, M., Camitta, B. M., Gaynon, P. S., Winick, N. J., Reaman, G. H., & Carroll, W. L. (2012). Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: A report from the children's oncology group. *Journal of Clinical Oncology*, *30*, 1663–1669. https://doi.org/10.1200/JCO.2011.37.8018

Jackmann, N., Mäkitie, O., Harila-Saari, A., Gustafsson, J., Nezirevic Dernroth, D., & Frisk, P. (2020). Vitamin D status in children with leukemia, its predictors, and association with outcome. *Pediatric Blood & Cancer*, *67*(4), e28163. https://doi.org/10.1002/pbc.28163

Karol, S. E., Yang, W., Van Driest, S. L., Chang, T. Y., Kaste, S., Bowton, E., Basford, M., Bastarache, L., Roden, D. M., Denny,

J. C., Larsen, E., Winick, N., Carroll, W. L., Cheng, C., Pei, D., Fernandez, C. A., Liu, C., Smith, C., Loh, M. L., … Relling, M. V. (2015). Genetics of glucocorticoid-associated osteonecrosis in children with acute lymphoblastic leukemia. *Blood*, *126*, 1770–1776. https://doi.org/10.1182/blood-2015-05-643601

Kawedia, J. D., Kaste, S. C., Pei, D., Panetta, J. C., Cai, X., Cheng, C., Neale, G., Howard, S. C., Evans, W. E., Pui, C.-H., & Relling, M. V. (2011). Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. *Blood*, *117*, 2340–2347. https://doi.org/10.1182/blood-2010-10-311969

Kerr Whitfield, G., Remus, L. S., Jurutka, P. W., Zitzer, H., Oza, A. K., Dang, H. T. L., Haussler, C. A., Galligan, M. A., Thatcher, M. L., Dominguez, C. E., & Haussler, M. R. (2001). Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Molecular and Cellular Endocrinology*, *177*, 145–149. https://doi.org/10.1016/s0303-7207(01)00406-3

Kim, H., Cho, C., Cho, Y., Cho, S., Yoon, K., & Kim, K. (2011). Significant associations of PAI-1 genetic polymorphisms with osteonecrosis of the femoral head. *BMC Musculoskeletal Disorders*, *12*, 160. https://doi.org/10.1186/1471-2474-12-160

Ladas, E. J., Orjuela, M., Stevenson, K., Cole, P. D., Lin, M., Athale, U. H., Clavell, L. A., Leclerc, J. M., Michon, B., Schorin, M. A., Welch, J. G., Asselin, B. L., Sallan, S. E., Silverman, L. B., & Kelly, K. M. (2016). Dietary intake and childhood leukemia: The diet and acute lymphoblastic leukemia treatment (DALLT) cohort study. *Nutrition*, *32*(10), 1103–1109.e1. https://doi.org/10.1016/j.nut.2016.03.014

Lee, Y. J., Lee, J. S., Kang, E. H., Lee, Y.-K., Kim, S.-Y., Song, Y. W., & Koo, K.-H. (2012). Vascular endothelial growth factor polymorphisms in patients with steroid-induced femoral head osteonecrosis. *Journal of Orthopaedic Research*, *30*, 21–27. https://doi.org/10.1002/jor.21492

Mattano, L. A. Jr., Devidas, M., Nachman, J. B., Sather, H. N., Hunger, S. P., Steinherz, P. G., Gaynon, P. S., & Seibel, N. L.; Children's Oncology Group. (2012). Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukemia: Results from the CCG-1961 randomized cohort trial. *The Lancet Oncology*, *13*, 906–915. https://doi.org/10.1016/S1470-2045(12)70274-7

Mattano, L. A. Jr., Sather, H. N., Trigg, M. E., & Nachman, J. B. (2000). Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: A report from the Children's Cancer Group. *Journal of Clinical Oncology*, *18*, 3262–3272. https://doi.org/10.1200/JCO.2000.18.18.3262

Mory, D. B., Rocco, E. R., Miranda, W. L., Kasamatsu, T., Crispim, F., & Dib, S. A. (2009). Prevalence of vitamin D receptor gene polymorphisms FokI and BsmI in Brazilian individuals with type1 diabetes and their relation to β-cell autoimmunity and to remaining β-cell function. *Human Immunology*, *70*, 447–451. https://doi.org/10.1016/j.humimm.2009.03.007

Niinimäki, R. A., Harila-Saari, A. H., Jartti, A. E., Seuri, R. M., Riikonen, P. V., Pääkkö, E. L., Möttönen, M. I., & Lanning, M. (2007). High body mass index increases the risk for osteonecrosis in children with acute lymphoblastic leukemia. *Journal of Clinical Oncology*, *25*, 1498–1504. https://doi.org/10.1200/JCO.2006.06.2539

Ojala, A. E., Pääkkö, E., Lanning, F. P., & Lanning, M. (1999). Osteonecrosis during the treatment of childhood acute lymphoblastic leukemia: A prospective MRI study. *Medical and Pediatric Oncology*, *32*, 11–17.

Padhye, B., Dalla-Pozza, L., Little, D., & Munns, C. (2016). Incidence and outcome of osteonecrosis in children and adolescents after intensive therapy for acute lymphoblastic leukemia (ALL). *Cancer Medicine*, *5*, 960–967. https://doi.org/10.1002/cam4.645

Plesa, M., Gagné, V., Glisovic, S., Younan, M., Sharif-Askari, B., Laverdière, C., Alos, N., Leclerc, J.-M., Sallan, S. E., Neuberg, D., Kutok, J. L., Silverman, L. B., Sinnett, D., & Krajinovic, M. (2019). Influence of BCL2L11 polymorphism on osteonecrosis during treatment of childhood acute lymphoblastic leukemia. *The Pharmacogenomics Journal*, *19*(1), 33–41. https://doi.org/10.1038/s41397-017-0002-4

Pui, C.-H., Campana, D., Pei, D., Bowman, W. P., Sandlund, J. T., Kaste, S. C., Ribeiro, R. C., Rubnitz, J. E., Raimondi, S. C., Onciu, M., Coustan-Smith, E., Kun, L. E., Jeha, S., Cheng, C., Howard, S. C., Simmons, V., Bayles, A., Metzger, M. L., Boyett, J. M., … Relling, M. V. (2009). Treating childhood acute lymphoblastic leukemia without cranial irradiation. *New England Journal of Medicine*, *360*, 2730–2741. https://doi.org/10.1056/NEJMoa0900386

Reisi, N., Iravani, P., Raeissi, P., & Kelishadi, R. (2015). Vitamin D and bone minerals status in the long-term survivors of childhood acute lymphoblastic leukemia. *International Journal of Preventive Medicine*, *6*(1), 87. https://doi.org/10.4103/2008-7802.164691. PMID: 26445634.

Relling, M. V., Yang, W., Das, S., Cook, E. H., Rosner, G. L., Neel, M., Howard, S., Ribeiro, R., Sandlund, J. T., Pui, C.-H., & Kaste, S. C. (2004). Pharmacogenetic risk factors for osteonecrosis of the hip among children with leukemia. *Journal of Clinical Oncology*, *22*, 3930–3936. https://doi.org/10.1200/JCO.2004.11.020

Rhodes, A., Gray, J., Harvey, N., Davies, J. H., Oreffo, R. O., Reading, I., Clarke, N. M., & Aarvold, A. (2017). Osteonecrosis following treatment for childhood acute lymphoblastic leukemia: The Southampton Children's Hospital experience. *Journal of Children's Orthopaedics*, *11*, 440–447. https://doi.org/10.1302/1863-2548.11.170142

Sakamoto, K., Imamura, T., Kihira, K., Suzuki, K., Ishida, H., Morita, H., Kanno, M., Mori, T., Hiramatsu, H., Matsubara, K., Terui, K., Takahashi, Y., Suenobu, S.-I., Hasegawa, D., Kosaka, Y., Kato, K., Moriya-Saito, A., Sato, A., Kawasaki, H., … Horibe, K. (2018). Low incidence of osteonecrosis in childhood acute lymphoblastic leukemia treated with ALL-97 and ALL-02 Study of Japan Association of Childhood Leukemia Study Group. *Journal of Clinical Oncology*, *20*(36), 900–907. https://doi.org/10.1200/JCO.2017.75.5066

Schrappe, M., Möricke, A., Reiter, A., Henze, G., Welte, K., Gadner, H., Ludwig, W.-D., Ritter, J., Harbott, J., Mann, G., Klingebiel, T., Gruhn, B., Niemeyer, C., Kremens, B., Niggli, F., Debatin, K.-M., Ratei, R., Stanulla, M., Beier, R., … Zimmermann, M. (2013). Key treatment questions in childhood acute lymphoblastic leukemia: Results in 5 consecutive trials performed by the ALL-BFM study group from 1981 to 2000. *Klinische Padiatrie*, *225*, S62–S72. https://doi.org/10.1055/s-0033-1337966

Segui, R., Estelles, A., Mira, Y., Espana, F., Villa, P., Falco, C., Vaya, A., Grancha, S., Ferrando, F., & Aznar, J. (2000). PAI-1 promoter 4G/5G genotype as an additional risk factor for venous thrombosis in subjects with genetic thrombophilic defects. *British Journal of Haematology*, *111*, 122–128. https://doi.org/10.1046/j.1365-2141.2000.02321.x

Seibel, N. L., Steinherz, P. G., Sather, H. N., Nachman, J. B., DeLaat, C., Ettinger, L. J., Freyer, D. R., Mattano, L. A., Hastings, C. A., Rubin,

C. M., Bertolone, K., Franklin, J. L., Heerema, N. A., Mitchell, T. L., Pyesmany, A. F., La, M. K., Edens, C., & Gaynon, P. S. (2008). Early postinduction intensification therapy improves survival for children and adolescents with high-risk acute lymphoblastic leukemia: A report from the Children's Oncology Group. *Blood*, *111*, 2548–2555. https://doi.org/10.1182/blood-2007-02-070342

Strauss, A. J., Su, J. T., Dalton, V. M., Gelber, R. D., Sallan, S. E., & Silverman, L. B. (2001). Bony morbidity in children treated for acute lymphoblastic leukemia. *Journal of Clinical Oncology*, *19*, 3066–3072. https://doi.org/10.1200/JCO.2001.19.12.3066

Te Winkel, M. L., Pieters, R., Hop, W. C. J., de Groot-Kruseman, H. A., Lequin, M. H., van der Sluis, I. M., Bökkerink, J. P. M., Leeuw, J. A., Bruin, M. C. A., Egeler, R. M., Veerman, A. J. P., & van den Heuvel-Eibrink, M. M. (2011). Prospective study on incidence, risk factors, and long-term outcome of osteonecrosis in pediatric acute lymphoblastic leukemia. *Journal of Clinical Oncology*, *29*, 4143–4150. https://doi.org/10.1200/JCO.2011.37.3217

Te Winkel, M. L., Pieters, R., Hop, W. C. J., Roos, J. C., Bökkerink, J. P. M., Leeuw, J. A., Bruin, M. C. A., Kollen, W. J. W., Veerman, A. J. P., de Groot-Kruseman, H. A., van der Sluis, I. M., & van den Heuvel-Eibrink, M. M. (2014). Bone mineral density at diagnosis determines fracture rate in children with acute lymphoblastic leukemia treated according to the DCOG-ALL9 protocol. *Bone*, *59*, 223–228. https://doi.org/10.1016/j.bone.2013.11.017

Zhang, Y., Wang, R., Li, S., Kong, X., Wang, Z., Chen, W., & Lin, N. A. (2013). Genetic polymorphisms in plasminogen activator inhibitor-1 predict susceptibility to steroid-induced osteonecrosis of the femoral head in Chinese population. *Diagnostic Pathology*, *8*, 169. https://doi.org/10.1186/1746-1596-8-169

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