ORIGINAL ARTICLE

Alterations of bone mineral metabolism of children with different cell lineage types of acute lymphoblastic leukaemia under chemotherapy

Tragiannidis A[1], Dokos Ch[1], Sidi V[3], Papageorgiou Th[1], Koliouskas D[3], Karamouzis M[4], Papastergiou Ch[5], Tsitouridis I[5], Katzos G[2], Rousso I[1], Athanassiadou-Piperopoulou F[1]

[1]2nd Pediatric Department, Hematology-Oncology Unit, AHEPA Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]1st Pediatric Department, Hippokratio Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]Pediatric Oncology Department, Hippokratio Hospital, Thessaloniki, Greece
[4]Laboratory of Clinical Biochemistry, AHEPA Hospital, Thessaloniki, Greece
[5]Radiology Department, Papageorgiou Hospital, Thessaloniki, Greece

Abstract

Background: Children with haematological malignancies such as acute lymphoblastic leukaemia (ALL) may have alteration of bone mineral metabolism therefore increased risk for osteopenia and osteoporosis.

Patients and Methods: The purpose of this study was to examine the alterations of bone mineral metabolism in two groups of children (*n*=42) according to immunophenotyping (B-cell type, T-cell type) both quantitative (bone mineral density *z*-scores) and qualitative (serum osteocalcin - OC and carboxyl-terminal telopeptide of human type I collagen - ICTP) during diagnosis (T=0), after the intensified chemotherapy period (T=0.5) and the consolidation period (T=1).

Results: According to our results 15 patients had osteopenia and 1 child developed osteoporosis at T=0.5 and 13 patients had osteopenia at T=1. Mean BMD *z*-score was significantly decreased in both groups during chemotherapy and especially statistically significant decline of T-cell type ALL group compared with B-cell type ALL patients. OC mean level remains in low levels for both groups reaching in plateau during chemotherapy and ICTP level was increased in T-cell type ALL group of patients compared with B-cell type in both periods of chemotherapy.

Conclusions: It seems that not only the combination of chemotherapeutic agents but also the cell lineage of ALL are important parameters of altering bone mineral metabolism. Hippokratia 2011; 15 (1): 43-47

Key words: acute lymphoblastic leukemia, bone mineral density, bone biochemical markers, children, cell lineage

Corresponding author: Athanasios Tragiannidis, Hematology-Oncology Unit, 2nd Pediatric Department, AHEPA Hospital, Aristotle University of Thessaloniki, 1, Kyriakidi Str, 54636, Thessaloniki, Greece, Tel: 0030 2310993944, Fax: 0030 230211131, e-mail: atragian@hotmail.com, atriagia@auth.gr

Over the recent years, life expectancy of children with cancer defined as event-free survival (EFS) and overall survival (OS) has increased. However studies indicate that chemotherapy protocols may have short term and late effects. In paediatric cancer patients, intensive chemotherapy and / or radiotherapy have many side effects most of which are identified by current literature. Endocrine deficiencies, renal failure, pulmonary and cardiac toxicity, obesity, osteopenia/osteoporosis are among the large group of short and long term effects[1-6].

Disturbances of bone metabolism and decreased bone mineral density (BMD) are known to frequently occur in childhood haematological malignancies and especially acute lymphoblastic leukaemia (ALL). Children with low BMD may are at increased risk of fracture and long term bone metabolism disturbances. Some studies indicate that antineoplastic agents such as corticosteroids, methotrexate and others may reduce BMD and alter bone mineral metabolism[3,7,8]. However, few studies have thoroughly examined the consequences of each phase of chemotherapy in different types of ALL patients (B-cell and T-cell lineage types) upon bone metabolism.

The present study examines bone mineral metabolism alterations in children with different types of ALL at various periods under chemotherapy.

Patients and methods

Patient selection

Forty two (42) children with ALL were treated according to the ALL Berlin Frankfurt Munster '95 (ALL BFM '95) chemotherapy protocol. Patients were admitted to the Haematology-Oncology Unit of the 2nd Paediatric Department of Aristotle University of Thessaloniki and Paediatric Oncology Department, Hippokratio Hospital of Thessaloniki for further evaluation and treatment. This study was conducted according to the Declaration of Helsinki (1964) and with written consent by all patients' parents. None of the patients was noted to suffer from

congenital or acquired disorder during diagnosis or have received any drug affecting bone mineral metabolism.

Methodology

Each child was grouped as standard/intermediate or high risk according to several criteria indicated by ALL BFM '95 protocol, Standard/intermediate group of children (B-cell lineage type ALL) received prednisone, vincristine, daunorubicin, L-asparaginase, cyclophosphamide, cytosine arabinoside, 6-mercaptopurine, thioguanine, high-dose methotrexate, doxorubicin, and dexamethasone, and in the high risk group (T-cell lineage type ALL) additional vindesine, ifosfamide, and etoposide.

For each patient demographic data and family/personal history were registered at diagnosis. All assessments were performed at diagnosis (T=0), at 6 months after initiation of intensive chemotherapy (after completion of induction/reinduction treatment) (T=0.5) and one year after diagnosis (during consolidation treatment) (T=1). At each time period, part from child physical examination, femoral neck BMD (g/m^2) was measured by dual energy x-ray absorptiometry (DEXA) (Norland® bone densitometer) and z-scores were standardized according to age and sex. *Z*-scores were estimated in each time periods and grouped into three major classes according to WHO criteria: (a) z-score <-1 is considered to be normal, (b) z-score from -1 - -2.5 indicates osteopenia and (c) z-score >-2.5 indicates osteoporosis[1,3].

Furthermore, blood samples by each child were taken for each time period. Serum levels of osteocalcin (OC; ng/ml) and carboxyl-terminal telopeptide of human type I collagen (ICTP; μg/l) were estimated with radio-immunoassays methods (RIA). Taking into account the age variation of the patients, normal values for this study were considered to be 60-70 ng/ml for serum OC according to a large survey with normal subjects by Cioffi M et al[9]. The highest normal limit of 11.5 μg/l for serum ICTP was set up according to the manufacturer's guidelines.

Results were evaluated according to the statistical program SPSS (version 13.0). For each parameter mean and standard error of the mean was calculated. A t-test for paired observations was performed for data at 0, 0.5 and 1. Statistical significance was set to $p < 0.05$.

Results

Mean age of patients was 5.92 ± 3.3 years (range: 2-13 years) at diagnosis. Among the 42 patients 24 (57.1%) were boys and 18 (42.9%) girls. According to immunophenotyping during diagnosis, 36 children (85.7%) had B-cell lineage ALL and 6 (14,3%) had T-cell lineage ALL. Among 42 children with ALL, 10 had osteopenia at the time of diagnosis (T=0), 15 had osteopenia and 1 developed osteoporosis at T=0.5 and 13 had osteopenia at T=1.

BMD *z*-scores, serum OC and ICTP means were recorded at time periods T=0, T=0.5 and T=1 and classified according to T-cell lineage ALL and B-cell lineage ALL (Table 1). At T=0, B-cell ALL patients had lower BMD *z*-score than T-cell ALL patients. However at T= 0.5, T-cell ALL patients had statistically significant lower BMD *z*-score (*z*-score -1.63) as compared with B-cell ALL children (*z*-score -0.697) (p = 0.02). Likewise at T=1, T-cell ALL children had statistically significant lower BMD z-score (*z*-score -1.20) as compared with B-cell ALL children (*z*-score -0.458) (p = 0.05). For all periods serum ICTP mean levels were much higher in T-cell ALL children than B-cell ALL ones, except for T=0.5 period when both groups had similar mean levels. At T=0 serum OC mean is much higher at B-cell type ALL patients than T-cell ALL children. Serum OC mean level of T-cell ALL patients, at T=0.5 period, was higher than that of the other group, however statistically significant higher levels were attained (53.266 vs. 39.305) only at T=1 period (p = 0.03).

Table 1: BMD z-score, serum OC and ICTP data for different chemotherapy periods

	T-cell ALL (mean ± SD)	B-cell ALL (mean ± SD)	p
BMD z-score (T=0)	-0.145 ± 0.366	-0.503 ± 0.774	NS
BMD z-score (T=0.5)	-1.63 ± 0.707	-0.697 ± 0.847	0.02
BMD z-score (T=1)	-1.20 ± 0.738	-0.458 ± 0.685	0.05
ICTP (ig/l, T=0)	12.046 ± 2.442	10.052 ± 4.215	NS
ICTP (ig/l, T=0.5)	16.196 ± 8.603	16.626 ± 5.209	NS
ICTP (ig/l, T=1)	15.298 ± 5.36	14.766 ± 5.135	NS
OC (ng/ml, T=0)	12.5 ± 5.192	14.377 ± 5.232	NS
OC (ng/ml, T=0.5)	47.416 ± 9.338	42.151 ± 17.795	NS
OC (ng/ml, T=1)	53.266 ± 12.284	39.305 ± 15.913	0.03

Mean BMD *z*-scores curves have the same pattern of decay for both groups of patients at T=0.5 and T=1 periods (Figure 1). Mean BMD z-scores for both groups reveal a significant decline of BMD over the period of

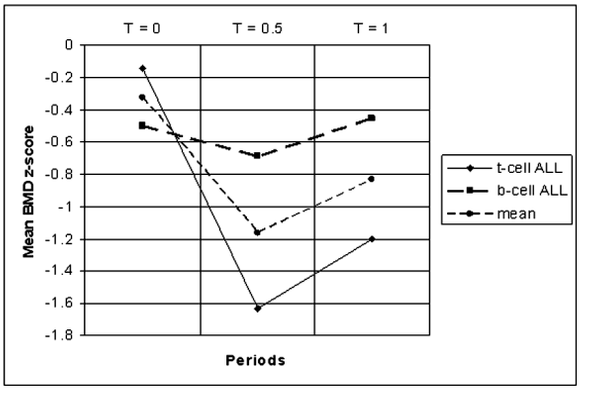

Figure 1: Alterations of mean BMD *z*-score at different periods of chemotherapy for T-cell and B-cell ALL patients.

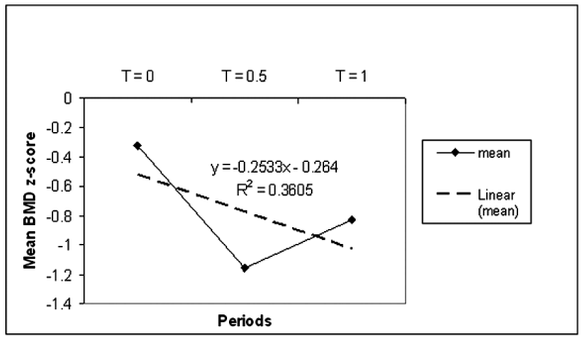

Figure 2: Mean BMD z-score curve during chemotherapy.

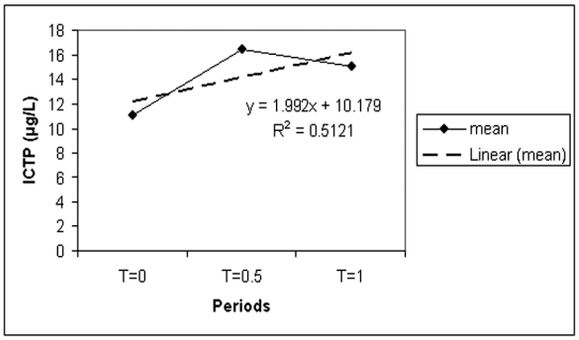

Figure 4: Mean ICTP curve during chemotherapy.

chemotherapy, as indicated by the gradient of the curve. Maximum decline is observed at T=0.5 period (Figure 2). Increased serum ITCP mean levels were recorded at T=0.5 and T=1 for both groups of patients. (Figure 3). ICTP mean level was increased at T=0.5 for both groups and had an increased gradient (Figure 4). T-cell ALL patients had higher levels of OC for periods T=0.5 and T=1 was compared with B-cell ALL children. An important increase of OC mean level as observed at T=0.5 for both groups as well as slightly increases at T=1 (Figure 5).

Discussion

Childhood haematological malignancies and especially ALL have rather unpleasant side-effects in bone mineral metabolism with increased risk of fracture. Our data confirm that ALL and chemotherapy contribute to bone mineral metabolism alterations as it has also been demonstrated by several reports in the current bibliography[10-12]. Low BMD *z*-scores at both T=0.5 and T=1 indicate that chemotherapy is an important factor for increase in the incidence of osteopenia/osteoporosis. According to our results 15 patients had osteopenia and 1 child developed osteoporosis at T=0.5 and 13 patients had osteopenia at T=1.

Statistically significant lower BMD *z*-scores were observed in the T-cell ALL group as compared with B-cell ALL group during the intensive period of chemotherapy and also the consolidation treatment. Only at diagnosis did children with B-cell ALL have lower BMD z-scores. This data confirm, in a way, the previous extensive study by Van der Sluis IM et al which informed that during diagnosis, low risk patients had lower BMD of the lumbar spine as compared to high-risk patients[13]. However T-cell ALL children have poorer prognosis and according to ALL BFM '95 chemotherapy protocol are classified into the high-risk group, receiving not only the classical combination of chemotherapy but also vindesine, ifosfamide, and etoposide. During chemotherapy T-cell ALL children had lower BMD *z*-scores as compared with B-cell ALL children, therefore presenting with an increased risk of bone fracture as confirmed by several studies[13].

Antineoplasmatic agents seem to reduce BMD during the early stages of chemotherapy but not in the same pattern during consolidation. According to our results, during consolidation the BMD *z*-score mean is much lower than at the time of diagnosis, in contrast with the lowest BMD *z*-score recorded during the intensive chemotherapy period. Several studies indicate that after intensive chemotherapy period, bone microarchitecture deterioration is a vital process responsible for the increased incidence of osteopenia, osteoporosis and fracture[10-12,14]. Microarchitecture deterioration does not exactly reflect low BMD *z*-scores value, as bone mineral density is a quantitative bone surface marker and not qualitative bone remodelling biomarker[7,11,13].

In the quest for a sensitive bone remodelling biomarker, serum ICTP and OC were estimated for both groups of patients at different periods of chemotherapy.

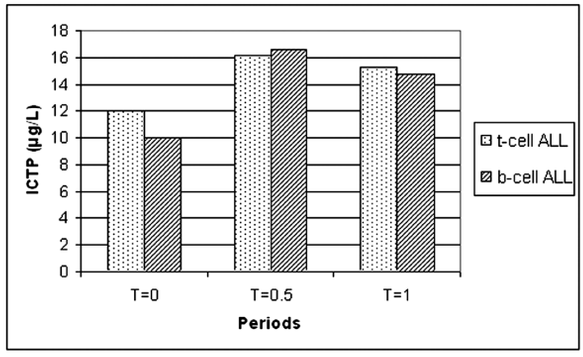

Figure 3: Mean ICTP at different periods of chemotherapy T-cell and B-cell ALL patients.

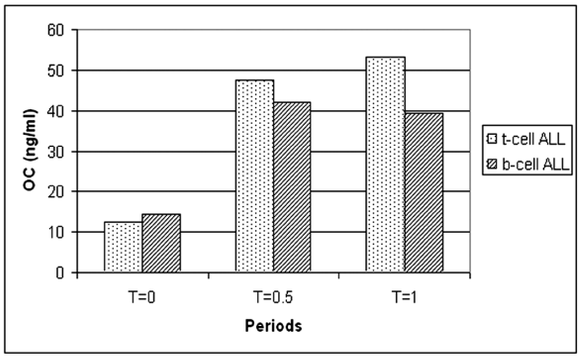

Figure 5: Mean OC at different periods of chemotherapy T-cell and B-cell ALL patients.

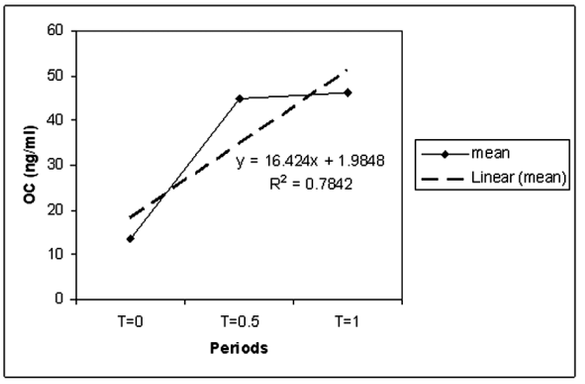

Figure 6: Mean OC curve during chemotherapy.

ICTP is increased at osteopenia-osteporosis states and it is widely used in the assessment of bone mineral metabolism alterations. Our data confirm that chemotherapy treatment period in ALL induces bone demineralization with subsequent increase in the levels of serum ICTP. Especially during the intensive chemotherapy period (T=0.5), ICTP was dramatically increased as compared with the consolidation period. Taking into account that mean ICTP level in T-cell ALL is higher than in B-cell ALL ones, even at diagnosis, cell lineage seems to be an important parameter for bone metabolism alterations.

Bone metabolism may be impaired by several serum factors secreted by leukemic cells such as the osteoblast-inhibiting factor and parathyroid hormone-related peptide. Studies indicate that leukemic cells infiltrate directly into bone and expand in the bone marrow spaces. This may lead to destruction of spongiosa, however no correlation between leukocyte count at diagnosis and markers of bone formation has been demonstrated according to previous studies[11,12,15,16]. During treatment of children with chemotherapy alone there is suppression of serum markers of bone formation coupled with a reduction of BMD[11-13,17]. In our study OC level, a sensitive biomarker of bone formation was lower than the normal mean level for age, indicating that chemotherapy alters bone mineral metabolism. Surprisingly T-cell ALL patients' OC mean level was higher at both periods (T=0.5 and T=1) as compared with the B cell group. It is of note that there is a statistically significant higher mean serum OC level in the T-cell ALL group as compared to the B-cell ALL group at T=0.5 period of chemotherapy. As expected the T-cell ALL group had lower OC mean levels as compared with B-cell ALL group because of more intensive chemotherapy. It can be hypothesized that during this period of intensified chemotherapy (T=0.5), mainly osteoblasts are affected in a gradual rather than in an abrupt manner. At consolidation the process of bone formation seems to play a major role in bone mineral homeostasis. However, we believe that low BMD *z*-scores and high ICTP levels reflect a domination of osteoclasts over osteoblasts in both periods of chemotherapy.

Past clinical studies have indicated that children being administered high-dose combination chemotherapy are more likely to develop osteopenia and present with greater suppression of their serum markers of bone formation, when compared to those being given fewer agents at a reduced dosage[18-21]. However, recent studies imply that bone mineral reduction during chemotherapy is a dynamic process involving osteoblasts diminished by a depleted osteoprogenitor cell pool[11,14,20]. Serum OC level is below normal for ALL patients, indicating a decline in osteoblast activity however a plateau of activity is observed during chemotherapy[17]. In addition ICTP mean level is increased during the intensive chemotherapy period subsequently leading to a decrease during consolidation for both groups examined. It may be hypothesized that bone mineral metabolism in periods of intensified chemotherapy is under regulation by a differentiated phenotype osteoblast with capacities of differentiated types of leukemic cell

In conclusion, intensive and consolidation periods of chemotherapy reduce BMD *z*-scores, and increase ICTP and OC serum levels in children with ALL. However, different patterns of bone metabolism alterations are observed in T-cell ALL and B-cell ALL. These may reflect possible interactions between leukemic cells and osteoblasts-osteoclasts. Since in this study there is a small number of patients in the T-cell group, more extensive studies are needed with daily recording of both serum ICTP and OC level in both periods of chemotherapy. The combination of antineoplastic agents, their cumulative doses and cell-lineage type are important factors for bone mineral alterations in children with ALL during chemotherapy resulting in increased fracture risk.

References

1. Haddy T, Mosher R, Reaman G. Osteoporosis in survivors of acute lymphoblastic leukemia. The Oncologist. 2001; 6: 278–285.
2. Haddy TB, Adde MA, McCalla J, Domanski MJ, Datiles 3rd M, Meehan SC, et al. Late effects in long-term survivors of high-grade non-Hodgkin's lymphomas. J Clin Oncol. 1998; 16: 2070-2079.
3. Kaste SC. Skeletal toxicities of treatment in childen with cancer. Pediatr Blood Cancer. 2008; 50: 469-473.
4. Mosher RB, McCarthy BJ. Late effects in survivors of bone tumors. J Pediatr Oncol Nurs. 1998; 15: 72-84.
5. Sala A, Barr RD. Osteopenia and cancer in children and adolescents. Cancer. 2007; 109: 1420-1431.
6. Yamashita N, Tanaka H, Moriwake T, Nishiuchi R, Oda M, Seino Y. Analysis of linear growth in survivors of childhood acute lymphoblastic leukemia. J Bone Miner Metab. 2003; 21: 172-178.
7. Bianchi ML. Osteoporosis in children and adolescents. Bone. 2007; 41: 486-495.
8. Van Beek RD, Keizer-Schrama de SMPF, Hakvoort-Cammel FG, Van der Sluis IM, Krenning EP, Pieters R, et al. No difference between prednisolone and dexamethasone treatment in bone mineral density and growth in long term survivors of childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2006; 46: 88-93.
9. Cioffi ME, Molinari AM, Gazzerro P, Di Finizio B, Fratta M, Deufemia A, et al. Serum osteocalcin in 1643 healthy children. Clin Chem. 1997; 43: 543-545.

10. Athanassiadou F, Tragiannidis A, Rousso I, Katsos G, Kolioukas D, Papastergiou C, et al. Evaluation of bone metabolism in children with acute lymphoblastic leukemia after induction chemotherapy. Pediatric Hematology Oncology. 2005; 22: 285-289.
11. Davies JH, Evans BAJ, Jenney MEM, Gregory JW. Skeletal morbidity in childhood acute lymphoblastic leukemia. Clin Endocrinology. 2005; 63: 1-9.
12. Van der Sluis IM, van den Heuvel-Eibrink MM. Osteoporosis in children with cancer. Pediatr Blood Cancer. 2008; 50: 474-478.
13. Van der Sluis IM, van den Heuvel-Eibrink MM, Hõhlen K, Krenning EP, Keizer-Schrama de Muinck SMPF. Altered bone mineral density and body composition, and increased fracture risk in childhood acute lymphoblastic leukemia. J Pediatr. 2002; 141: 204-210.
14. Tragiannidis A, Athanassiadou F, Rousso I, Katzos G, Sidi V, Papageorgiou T, et al. Evaluation of bone metabolism after induction chemotherapy in children with acute lymphoblastic leukemia. Pediatr N Gr. 2004; 16: 62-67.
15. Cohn SL, Morgan ER, Malette LE. The spectrum of metabolic bone disease in lymphoblastic leukemia. Cancer. 1987; 59: 346-50.
16. Mundy GR, Luben RA, Raisz LG, Oppenheim JJ, Buell DN. Bone resorbing activity in supernatants from lymphoid cell lines. N Engl J Med. 1974; 290: 867-871.
17. Boot AM, van den Heuvel-Eibrink MM, Hõhlen K, Krenning EP, Keizer-Schrama de Muinck SMPF. Bone mineral density in children with acute lymphoblastic leukaemia. Eur J Cancer. 1999; 35: 1693-1697.
18. Crofton PM, Ahmed SF, Wade JC, Elmlinger MW, Ranke MB, Kelnar CJH, et al. Bone turnover and growth during and after continuing chemotherapy in children with acute lymphoblastic leukemia. Pediatric Research. 2002; 48: 490–496.
19. Crofton PM, Ahmed SF, Wade JC, Elmlinger MW, Ranke MB, Kelnar CJH, et al. Effects of a third intensification block of chemotherapy on bone and collagen turnover, insulin growth factor I, its binding proteins and short-term growth in children with acute lymphoblastic leukemia. Eur J Cancer. 1999; 35: 960-967.
20. Davies JH, Evans BAJ, Jenney MEM, Gregory JW. In vitro effects of chemotherapeutic agents on human osteoblast-like cells. Calcif Tissue Int. 2002; 70: 408-415.
21. Scheven BAA, van der Veen MJ, Damen CA, Lafeber FPJG, van Rijn HJM, Bijlsma JWJ, et al. Effects of methotrexate on human osteoblasts in vitro: modulation by 1,25-dihydroxyvitamin D3. J Bone Miner Res. 1995; 10: 874-880.