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Selective reduction in trabecular volumetric bone mineral density during treatment for childhood acute lymphoblastic leukemia

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ABSTRACT

During treatment of childhood acute lymphoblastic leukemia (ALL) fracture incidence is increased. Studies using DXA, which measures a composite of both trabecular and cortical BMD, have shown reduced BMD during treatment. We investigated changes in compartmental (cortical and trabecular) volumetric BMD (vBMD) and bone geometry using peripheral quantitative computed tomography. These outcomes were also analysed in relation to adiposity and treatment factors. Thirty nine patients with ALL (64% male, median age 7.2 years (4.1–16.9)) were compared to 34 healthy controls (50% male, median age 9.1 years (4.4–18.7)). DXA-derived age-specific standard deviation scores (SDS) of the lumbar spine (LS) and femoral neck (FN) were reduced in subjects with ALL compared to controls ($p \leq 0.01$). This persisted following adjustment for body size using height-specific SDS (LS -0.72 ± 1.02 vs -0.18 ± 0.72, $p = 0.01$; FN -1.53 ± 0.96 vs -0.74 ± 0.74, $p = 0.001$) and bone mineral apparent density (BMAD) SDS (LS -0.76 ± 1.14 vs 0.04 ± 1.08, $p = 0.01$; FN -1.63 ± 1.38 vs -0.16 ± 1.20, $p < 0.001$). Radial and tibial trabecular vBMD was also reduced (196.5 ± 54.9 mg/cm^3 vs 215.2 ± 39.9 mg/cm^3, $p = 0.03$ and 232.8 ± 60.3 mg/cm^3 vs 267.5 ± 60.2 mg/cm^3, $p = 0.002$, respectively), but cortical vBMD at the radius and tibia was similar in patients and controls. A lowered tibial bone strength index (BSI) was identified in patients with ALL (53.9 ± 23.1 mg/mm^4 vs 82.5 ± 27.8 mg/mm^4, $p < 0.001$) suggesting lower fracture threshold from compressive forces. No relationships with measures of adiposity, duration of treatment or cumulative corticosteroid dose were identified. Our findings therefore suggest that reduction in trabecular vBMD during childhood ALL treatment may contribute to the observed increased fracture incidence and bony morbidity in this group.

Introduction

An increase in fracture incidence is observed throughout the treatment course for childhood acute lymphoblastic leukemia (ALL), and modern treatment regimens have not altered this outcome [1–3]. The aetiology is multi-factorial and includes the leukemic disease process and administration of chemotherapy and glucocorticoids. A recent study demonstrated a doubling of expected fracture incidence during treatment for ALL, and fractures were most frequent following the initial induction and intensification phases of treatment, when multi-agent, high-dose chemotherapy had been administered [1]. Others have also shown increased fracture rates, and that the reduction in bone mineral density (BMD) was greatest following the first 6 months of treatment, with little recovery during the less intensive maintenance phase of treatment [4–6].

Whilst reduced areal BMD is associated with increased fracture risk in children [3,7] the mechanical strength of bone is determined not only by the BMD, but also structural properties such as cross sectional area and cortical thickness. Unlike DXA, peripheral quantitative computed tomography (pQCT) is able to determine compartmental volumetric bone mineral density (vBMD), and additionally geometric properties of bone structure. Estimates of bone strength and resistance to torsion and compressive forces can be derived from pQCT scans, and have been correlated with likelihood of fracture ex vivo [8,9].

In two studies, reduced radial trabecular vBMD was associated with increased fracture risk in healthy adults and children [10,11], and has also been reported in a cohort of childhood ALL survivors

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who had completed treatment [12]. However, in this cohort, bone area was increased and axial moment of inertia, a measure of resistance to bending and torsion, was maintained compared to healthy controls, suggesting a possible adaptive mechanism to reduced BMD following treatment for ALL [12]. No previous studies have investigated these outcomes during treatment for childhood ALL.

Furthermore, evidence is accumulating that children with greater fat mass sustain more fractures, and have lower BMD [13,14]. It is well recognised that children with ALL have greater adiposity compared to healthy peers, and this may further contribute to negative musculoskeletal outcomes. Bone and adipose tissue interact through hormonal mechanisms. Leptin, an adipocyte-derived hormone, acts centrally via the hypothalamus and sympathetic nervous system to reduce osteoblast activity and bone formation [15]. Conversely, local leptin production by bone marrow adipocytes may act in a paracrine manner to increase osteoblast function. Adiponectin is also secreted by adipocytes, but production is inversely correlated with body fat. In vitro, adiponectin can increase human osteoblast proliferation [16]. Osteocalcin, an osteoblast derived hormone, acts on adipocytes to increase adiponectin secretion and decrease fat mass [17], thereby indirectly reducing leptin secretion.

The aims of this study were to evaluate volumetric BMD and bone geometry during treatment for childhood ALL. Additionally, the relationship of these outcomes to cumulative doses of chemotherapeutic agents, body composition and serum markers of the bone-fat feedback loop was evaluated.

Materials and methods

Study subjects

Children undergoing treatment for ALL at the regional paediatric oncology unit at Southampton University Hospitals NHS Trust, UK were invited to participate. Exclusion criteria included age $\leq$4 years at time of recruitment, relapse of ALL, previous osteonecrosis, bone marrow transplantation, treatment with cranial or other radiotherapy and additional significant chronic disease with or without long-term steroid therapy. All subjects were treated according to MRC UKALL 2003 treatment protocol. Briefly, this consists of a 6 month period of intensive multiagent chemotherapy, followed by a period of maintenance chemotherapy. The total duration of treatment with chemotherapy was 112 weeks for females and 164 weeks for males. The cumulative dose of glucocorticoid and methotrexate exposure during ALL treatment was obtained by retrospective case notes analysis. A healthy control group who had never received steroid treatment and had no other chronic medical conditions was recruited by asking subjects to invite a friend of similar age to participate. Fracture history was determined in both groups by direct questioning.

The study was approved by the Isle of Wight, Portsmouth and South East Research Ethics Committee and written informed consent was obtained from all participants and/or their parent or guardian.

Anthropometric assessment

Height was measured using a wall mounted stadiometer (Marsden HM-200) and weight to the nearest 0.1 kg using electronic scales (Marsden MPPS-250). Standard deviation scores (SDS) were calculated for height, weight and body mass index (BMI) from the 1990 British reference data [18,19].

Four-site skinfold thickness (triceps, biceps, subscapular, suprailiac) was performed on the non-dominant site of the body using a Holtain Skinfold Caliper (Holtain Ltd., Pembs, UK) by two researchers trained in this technique (R.L.S and L.D). Waist circumference was measured with a cloth tape at the midpoint between the subcostal margin and the iliac crest. SDS for triceps, subscapular and suprailiac skinfold thickness and waist circumference were calculated from National Health and Nutrition Examination Survey (NHANES II) data [20]. Fat percentage was calculated from skinfold thicknesses using published age-appropriate equations [21,22]. These equations are shown in Appendix 1.

Pubertal status was assessed by participant self-assessment using standard photographs and a Prader orchidometer. The subject (or parent for younger children) received instruction on how to measure testicular volume using the orchidometer before being asked to perform self (or by parent) examination in a private room. Pubertal staging was classified according to the method of Tanner.

Dual-energy X-ray Absorptiometry Scans (DXA)

Bone parameters were assessed using a Hologic Discovery W Dual-energy X-ray Absorptiometer (Hologic, Inc., Bedford, MA, USA) with fan beam technology (software version 12.5). DXA measurements were taken at the non-dominant femoral neck (FN) and lumbar spine (L1–L4 vertebrae) (LS). BMD, bone area and bone mineral content (BMC) were calculated. To minimize the effect of body size on areal BMD, a validated transformation was made of the DXA data to calculate a volumetric density (bone mineral apparent density (BMAD)) [23]. This uses the assumption that the measured site is a cylinder with a volume proportional to the second power of the projected anteroposterior area obtained from DXA measurement of areal BMD. SDS for participant age were calculated using published British reference data [23]. Height specific SDS were also calculated using published North American reference data as a further method of adjusting for body size [24].

Body composition data (fat mass and lean mass) were also obtained during the whole body DXA scan. Lean mass index was calculated using lean mass (kg)/height $(m)^2$.

Peripheral quantitative computed tomography (pQCT)

pQCT scanning was performed using a Stratec XCT-2000 scanner (Stratec Inc. Pforzhein, Germany). The non-dominant forearm and leg were scanned where possible, although in some ALL subjects this was prevented by the positioning of long-term vascular access lines. Two sites were scanned for each limb, a metaphyseal site (largely trabecular bone) and a diaphyseal site (largely cortical bone). For radial measurements, the metaphyseal (R4) and diaphyseal (R66) sites corresponded to 4% and 66% of the distance from the ulnar styloid process to the olecranon, respectively. Tibial metaphyseal (T4) and diaphyseal (T66) sites corresponded to 4% and 66% of the distance from the medial malleolus to the tibial tuberosity, respectively. At each site a single 2 mm thick tomographic slice was sampled at a voxel size of 0.5 mm.

At the metaphyseal site, bone mineral content (BMC), total vBMD (the mean mineral density of the total cross-section), trabecular vBMD and total bone cross-sectional area (CSA) were calculated using the manufacturer's software version 5.4. At the proximal site, BMC, total vBMD, cortical vBMD, total bone CSA, cortical CSA and muscle CSA were obtained. Cortical thickness was calculated using the circular ring model in which bone was assumed to be a cylinder [25]. The BMC:muscle ratio was calculated as an indicator of bone mineralisation relative to muscle strength [26].

Bone strength measurements were generated from the pQCT outputs. Torsional resistance was estimated by the strength-strain index (SSI) at the diaphyseal site of the radius. Bone-strength index (BSI) is calculated from measurements at the metaphyseal sites ([total bone density]^2 $\times$ total bone area) and provides an estimate of a bone's ability to withstand compression [8].

Adipokines

Serum samples were analysed for adiponectin, leptin and osteocalcin using commercially available ELISA assays (adiponectin and leptin: AssayPro, St Charles, MO, USA; osteocalcin: eBioscience,