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Original Paper

Effects of a Third Intensification Block of Chemotherapy on Bone and Collagen Turnover, Insulin-like Growth Factor I, its Binding Proteins and Short-term Growth in Children with Acute Lymphoblastic Leukaemia

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Children with acute lymphoblastic leukaemia (ALL) have reduced bone turnover caused by the disease itself and early intensive chemotherapy, but the effects of later chemotherapy using different drug combinations are uncertain. We report here a longitudinal study on 9 children with ALL randomised to receive an additional third intensification block of chemotherapy, compared with 9 children receiving continuing chemotherapy over the same period. During third intensification, bone alkaline phosphatase, procollagen type I C-terminal propeptide, the carboxyterminal propeptide of type I collagen, procollagen type III N-terminal propeptide and lower leg length all decreased in response to dexamethasone, then returned to (but not beyond) baseline levels after dexamethasone was stopped and other drugs started. These changes were unrelated to circulating insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-3 or IGFBP-2. In all children, bone alkaline phosphatase remained below the population mean throughout. We conclude that dexamethasone decreased bone and soft tissue turnover, probably through direct effects on target tissues. The postdexamethasone phase of third intensification and continuing chemotherapy had no major deleterious effect on collagen turnover, but there was evidence of continuing suboptimal bone mineralisation. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: children, acute lymphoblastic leukaemia, chemotherapy, bone turnover, growth, alkaline, phosphatase, collagen peptides, insulin-like growth factor I, insulin-like growth factor binding proteins
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INTRODUCTION

CHILDREN WITH acute lymphoblastic leukaemia (ALL) grow poorly during intensive chemotherapy but tend to catch-up during less intensive periods or after completion of chemotherapy [1–10]. Some experience bone pain and there have been reports of an increased incidence of fractures and reduced bone mineral content both during and after chemotherapy [11, 12], but the relative contributions from the disease itself and the many drugs used in its treatment have been unclear. For survivors, the risk of developing future osteoporosis is also unclear.

We have recently demonstrated that, at diagnosis, children with ALL have low bone turnover and are in a growth hormone (GH) resistant state [13]. During induction and early intensive chemotherapy, further suppression of osteoblast proliferation and osteoclast activity and lower leg growth occurred, probably largely through the direct action of prednisolone on bone [10, 13]. Those who were subsequently treated with high-dose intravenous methotrexate as central

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nervous system (CNS)-directed treatment had reduced bone formation and enhanced resorption compared with those who were not.

We report here a follow-up study on a small cohort of children who were later randomised to receive a third intensification block of chemotherapy lasting 8 weeks and comprising a different combination of drugs from those used in the first two intensifications, namely dexamethasone, asparaginase, vincristine, cytarabine, thioguanine and cyclophosphamide. We assessed the effects of this third intensification block on biochemical markers of bone and collagen turnover, insulin-like growth factor I (IGF-I), its binding proteins and short-term growth compared with those children who were randomised to receive standard continuing chemotherapy only.

PATIENTS AND METHODS

Patients

We studied 18 children with ALL during weeks 35–51 of chemotherapy. The children were already enrolled in the national Medical Research Council randomised trial of childhood ALL treatment, which was performed from 1992 to 1997 (UKALL XI-92). In this trial, all randomisations were carried out centrally; there was no local selection. All children presenting to the Royal Hospital for Sick Children, Edinburgh between January 1993 and June 1994 with a diagnosis of ALL were eligible to participate in our study and all agreed to do so. All had previously received induction chemotherapy, followed by two periods of intensification chemotherapy, as previously described [13] and all were in first clinical and haematological remission. 9 children were randomised to receive continuing chemotherapy only over weeks 35–51 (group 1) (Figure 1). One child in group 1 had previously received a 2 week course of cranial irradiation during weeks 10–11 of chemotherapy. The concentrations of all her markers were within the range of the other children in that group (in particular, her IGF-I level remained slightly above the population mean). The remaining 9 children were randomised to a third intensification chemotherapy block according to the national protocol (group 2). The median ages of the children were 3.8 years (range 2.0–9.1 years) and 5.1 years (range 2.8–14.3 years) in groups 1 and 2, respectively. There were 7 boys and 2 girls in each group. In group 1, 6 children had previously received high-dose intravenous (i.v.) methotrexate as CNS-directed treatment and 3 had intrathecal methotrexate only, as previously described [13]. In group 2, 5 children had received high-dose i.v. methotrexate as CNS-directed treatment and 4 had intrathecal methotrexate only. The study was approved by the local ethics committee and informed consent was obtained from parents and (where appropriate) children.

Drug protocols, samples and anthropometric measurements

Figure 1 illustrates the drug protocols for each group, together with the blood sampling intervals. Drug dosages for continuing chemotherapy were: prednisolone 40 mg/m^2; vin-

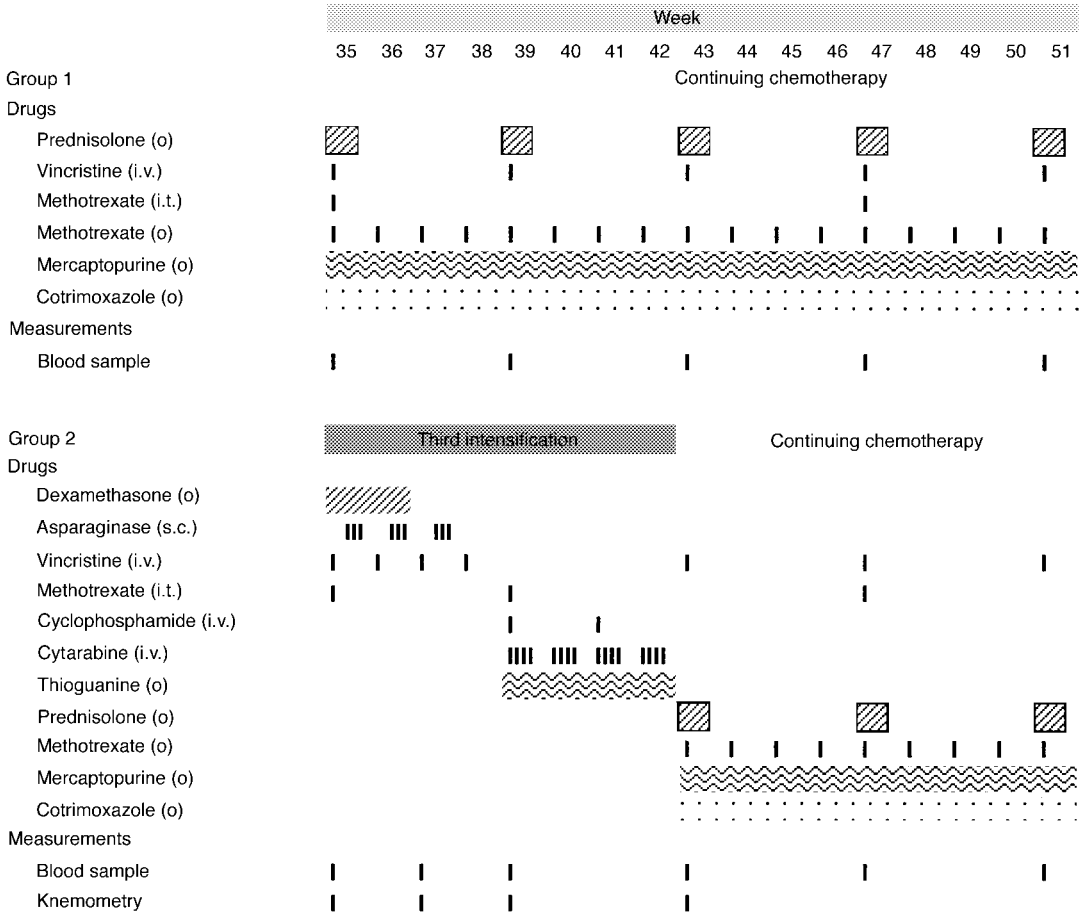

Figure 1. Schedule for drug administration and timing of measurements for group 1 and group 2. o, oral; s.c., subcutaneous; i.v., intravenous; i.t., intrathecal.

cristine 1.5 mg/m^2; intrathecal methotrexate 10 mg (age 2.0–2.9 years), 12.5 mg (age $\geq$3.0 years); oral methotrexate 20 mg/m^2; mercaptopurine 75 mg/m^2; cotrimoxazole 480 mg/day (body surface area 0.5–0.75 m^2), 720 mg/day (body surface area 0.76–1.0 m^2), 960 mg/day (body surface area $>$1.0 m^2). Drug dosages for third intensification were: dosages as above plus dexamethasone 10 mg/m^2/day; asparaginase 6000 u/m^2; cyclophosphamide 600 mg/m^2; cytarabine 75 mg/m^2; thioguanine 60 mg/m^2.

In group 2, the measurement at week 35 was taken immediately before third intensification began. Third intensification was from week 35 to week 42, after which the children followed the same continuing chemotherapy protocol as the children in group 1. In group 2, an extra blood sample was collected at week 37, immediately after dexamethasone had been stopped. In group 1 children this blood sample was omitted because venous access was only available at the time of vincristine administration and we felt that it would be unethical to perform venepuncture solely for this research study. All measurements shown in Figure 1 as coinciding with short (5 day) courses of prednisolone were taken immediately before the first dose of prednisolone was given. Samples were collected between 1100 h and 1500 h to minimise the effects of circadian variation. Plasma and serum were stored in aliquots at -70°C until analysis.

We measured lower leg length by knemometry in 4 children from group 2 who were able to cooperate. Measurements were made in the morning, using the random zero method [14] at the intervals shown in Figure 1. The median technical error (one standard deviation (S.D.) from the mean of a set of triplicate measurements) was 0.15 mm. No knemometry was performed on the children in group 1 because all but one were too young to cooperate in the technique.

Analytical methods

Collagen marker assays. We measured procollagen type I C-terminal propeptide (PICP), the cross-linked telopeptide of type I collagen (ICTP) and procollagen type III N-terminal propeptide (P3NP) in plasma by radioimmunoassay (RIA; Orion Diagnostica, Espoo, Finland), using methods previously described [15–17]. Before analysis, we diluted samples appropriately in 154 mmol/l sodium chloride to achieve concentrations within the calibration curve; typical dilutions were 1 in 4 for PICP and 1 in 2 for ICTP and P3NP. All samples were analysed in duplicate. As far as possible, samples from each patient were analysed in a single analytical run to minimise analytical variation. Between-run coefficients of variation were 7.8% and 5.2% at 94 μg/l and 320 μg/l for PICP, 6.3% and 9.2% at 8.7/μg/l and 33.8 μg/l for ICTP and 5.6% and 6.4% at 4.6 μg/l and 10.4 μg/l for P3NP.

Bone alkaline phosphatase. Bone alkaline phosphatase was measured in plasma by wheatgerm lectin affinity electrophoresis, as previously described [18]. Between-run coefficients of variation were 2.2%, 3.5% and 1.9% at 251, 349 and 435 U/l, respectively.

IGF-I. IGF-I was measured in serum with a specific RIA (Mediagnost, Tübingen, Germany). This assay uses an excess of IGF-II to eliminate interferences by IGF binding proteins (IGFBPs) [19]. Between-assay coefficients of variation were 8.5%, 6.5% and 8.0% at 69 μg/l, 140 μg/l and 118 μg/l, respectively.

IGFBP-3. IGFBP-3 was measured in serum using a specific RIA as described previously [20]. Between-assay coefficients of variation were 7.3% and 6.9% at 2772 μg/l and 3545 μg/l, respectively.

IGFBP-2. IGFBP-2 was measured in serum using a specific RIA [21]. Recombinant human IGFBP-2 (a gift from Sandoz, Basel, Switzerland) was used as a standard and as a tracer. The sensitivity of the assay was 0.2 μg/l. The between-assay coefficient of variation was 10.7%.

Data analysis

The distributions of serum concentrations of collagen markers, IGF-I and IGFBPs in healthy children are log-normal [22, 23]. We therefore transformed measured concentrations to their logarithms before calculating age- and sex-specific S.D. scores, based on our own published data [22, 23]. Bone alkaline phosphatase did not require log transformation; S.D. scores were calculated in relation to our own published data [24]. For the knemometry data, we calculated lower leg length velocity for each time point by subtracting from the lower leg length at that time point the length measured at the previous time point, and dividing by the exact time interval between the two measurements. The results were expressed as mm/week.

The mean and 95% confidence limits of the mean were calculated for each analyte at each time point. We employed non-parametric statistical tests throughout because variances were not always equal despite log transformation. Data were compared longitudinally through time using the Friedman test, then compared pairwise using Wilcoxon-signed rank tests; comparisons between groups were by Mann–Whitney *U* tests with correction for ties. Spearman rank correlations with correction for ties were used to compare variables. All statistical tests were two-tailed; $P < 0.05$ was regarded as significant.

RESULTS

Figure 2 shows the individual changes in the markers in each of the two groups over the period studied. The means and 95% confidence intervals (CI) for each group are presented in Figure 3. In group 1 there were no significant changes through time in any of the markers ($P > 0.11$). However, 1 individual in group 1 showed a marked decrease in bone alkaline phosphatase, PICP, ICTP and P3NP (but not IGF-I, IGFBP-3 or IGFBP-2) at week 39, coinciding with an episode of febrile neutropenia.

In group 2, we found significant changes through time for PICP, ICTP, IGFBP-2 and lower leg length velocity (LLLV) ($P < 0.05$), but changes in bone alkaline phosphatase ($P = 0.078$) and P3NP ($P = 0.083$) failed to reach significance for these small numbers. There was no significant change through time for IGF-I ($P = 0.51$) and IGFBP-3 ($P = 0.63$). Following dexamethasone administration, bone alkaline phosphatase, PICP, ICTP and P3NP decreased at week 37 in all patients except for 1 in whom collagen markers decreased but bone alkaline phosphatase increased slightly. The mean decreases in S.D. score (95% CI) from week 35 to week 37, for bone alkaline phosphatase, PICP, ICTP and P3NP, respectively, were -0.63 (-1.10, -0.17), -2.83 (-4.48, -1.19), -2.17 (-3.95, -0.38) and -3.01 (-4.57, -1.45) ($P < 0.05$; Figure 3a–d). The collagen markers had returned to predexamethasone levels by week 39 although bone alkaline phosphatase recovered slightly more slowly ($P < 0.05$); little further change in these markers was observed thereafter, although ICTP showed a fluctuating pattern. In contrast to

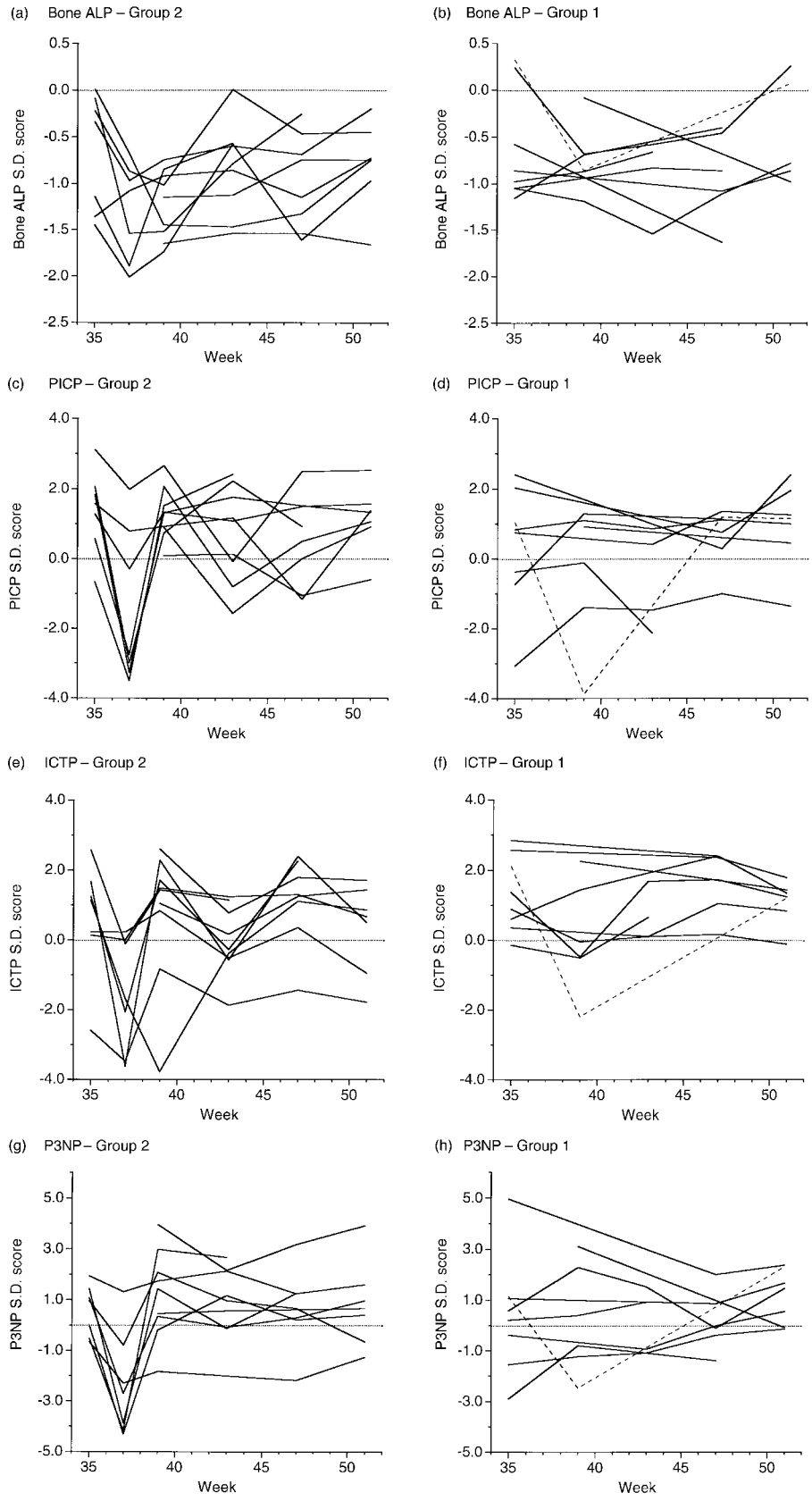

Figure 2. Changes in measurements in individual children over weeks 35–51 of chemotherapy. (a) Bone alkaline phosphatase (ALP), group 2, (b) bone alkaline phosphatase, group 1, (c) procollagen type 1 C-terminal propeptide (PICP), group 2, (d) PICP, group 1, (e) cross-linked telopeptide of type I collagen (ICTP), group 2, (f) ICTP, group 1, (g) procollagen type III N-terminal propeptide (P3NP), group 2, (h) P3NP, group 1, (i) insulin-like growth factor I (IGF-I), group 2, (j) IGF-I, group 1, (k) IGF binding protein 3 (IGFBP-3), group 2, (l) IGFBP-3, group 1, (m) IGFBP-2, group 2, (n) IGFBP-2, group 1, (o) lower leg length velocity (LLLV), group 2. The broken line indicates a child in group 1 who experienced an episode of febrile neutropenia around week 39.

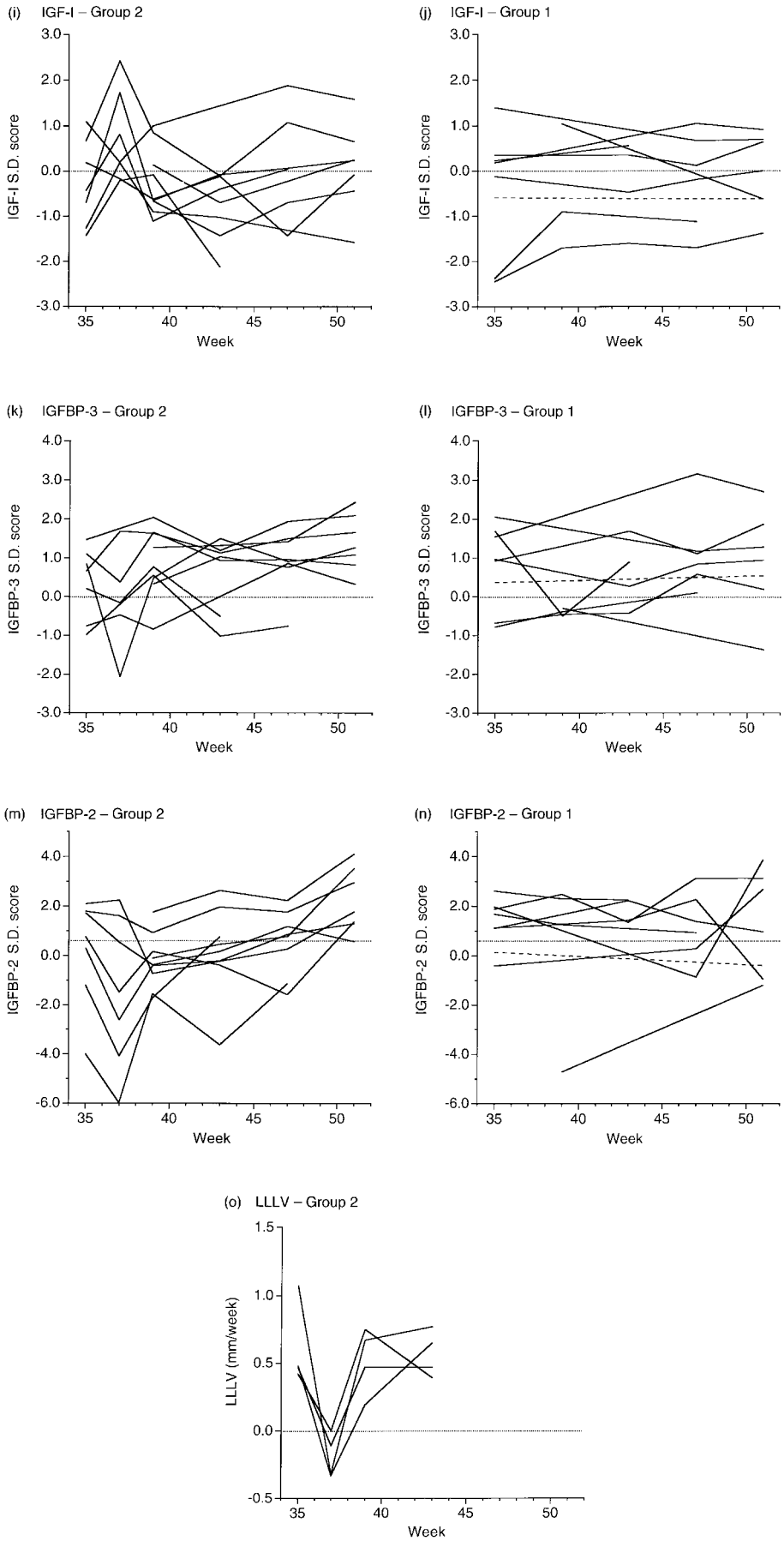

Figure 2. ***continued.***

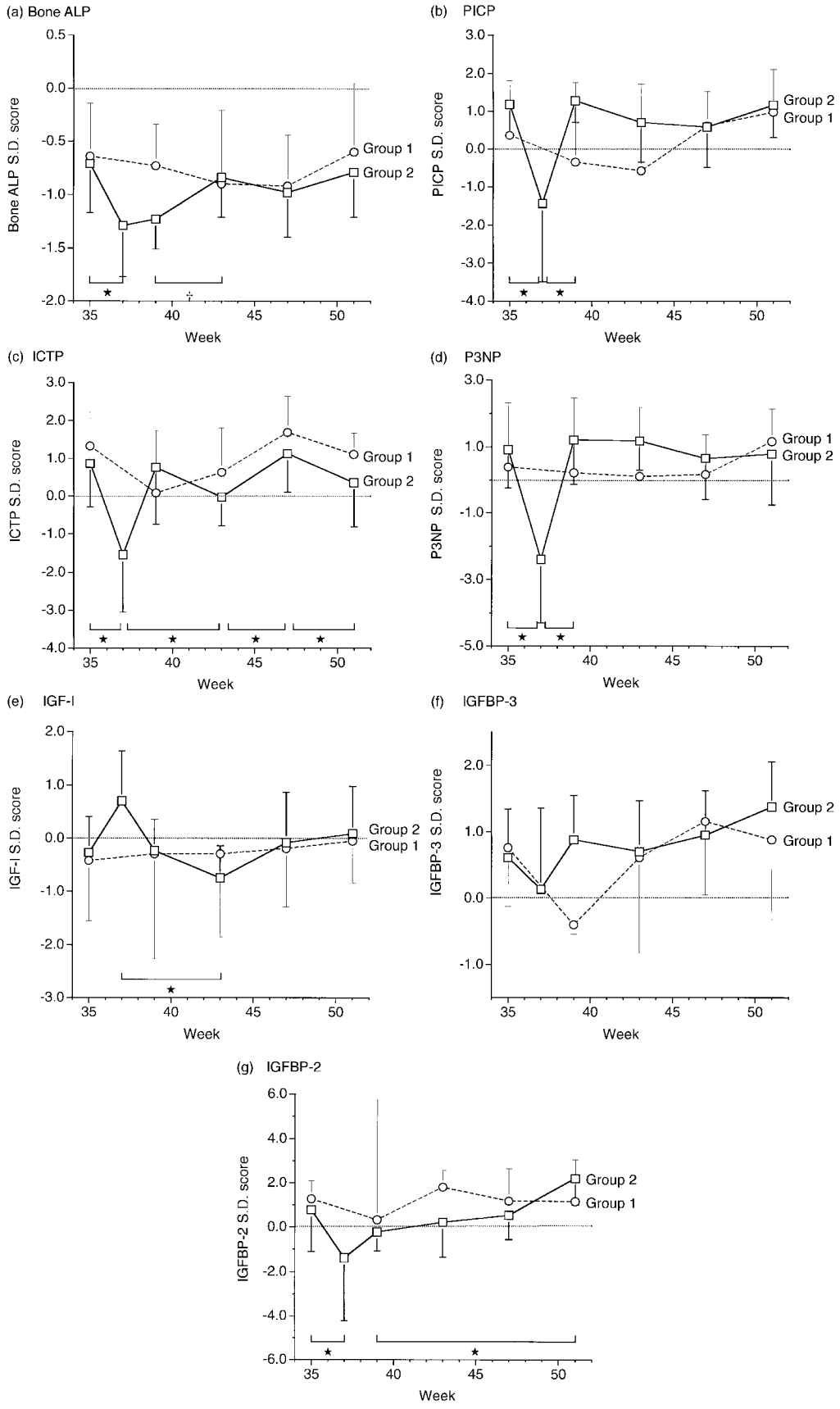

Figure 3. Changes in measurements in group 2 (squares) compared with group 1 (circles) over weeks 35–51 of chemotherapy. (a) Bone alkaline phosphatase (ALP), (b) procollagen type I C-terminal propeptide (PICP), (c) cross linked telopeptide of type I collagen (ICTP), (d) procollagen type III N-terminal propeptide (P3NP), (e) insulin-like growth factor I (IGF-I), (f) IGF binding protein (IGFBP-3), (g) IGFBP-2. *$P \leq 0.05$, †$P \leq 0.01$ for longitudinal comparisons in group 2.

the bone and collagen turnover markers in group 2, IGF-I (Figure 3e) and IGFBP-3 (Figure 3f) showed no consistent change at week 37, but IGFBP-2 (Figure 3g) decreased with a mean change in S.D. score (confidence intervals) of − 1.60 (−2.75, −0.46; $P < 0.05$) at week 37 before gradually returning to predexamethasone levels by week 51 ($P < 0.05$). There were few significant correlations among markers in group 2 at any time point. In the 4 children from group 2 who had knemometry, there was a decrease in LLLV in every case at week 37 (shrinkage of the lower leg was observed in 3 children), but lower leg growth subsequently returned to predexamethasone levels (Figure 2o).

Comparing group 1 with group 2 at week 35 and from weeks 39 to 51, differences between the groups did not achieve significance for PICP, ICTP, P3NP, IGF-I and IGFBP-2 ($P > 0.05$). However, at 39 weeks, bone alkaline phosphatase in group 2 was lower than in group 1 (Figure 3a) and IGFBP-3 (Figure 3f) in group 2 was higher than in group 1 ($P < 0.05$).

In groups 1 and 2 combined, at weeks 35 and 51 those children who had previously received high-dose methotrexate tended to have lower bone alkaline phosphatase activities and higher ICTP and P3NP concentrations than those who had not, but these differences did not achieve statistical significance ($P > 0.05$). At weeks 35 and 51, mean bone alkaline phosphatase S.D. scores were − 0.82 and − 0.82, respectively in the high-dose methotrexate group compared with − 0.46 and − 0.43 in the non high-dose group; mean ICTP S.D. scores were + 1.35 and + 1.04 compared with + 0.72 and − 0.04; and mean P3NP S.D. scores were + 1.19 and + 1.33 compared with -0.07 and + 0.12.

DISCUSSION

In a previous study of children with ALL, we showed that collagen markers of bone formation (PICP), bone resorption (ICTP) and soft tissue formation (P3NP) were all suppressed during induction and first intensification chemotherapy, probably mainly by prednisolone [13]. After prednisolone was stopped, the collagen markers and bone alkaline phosphatase showed a dramatic 'catch-up', coinciding with a resumption of lower leg growth, although bone alkaline phosphatase remained well below the population mean. We also demonstrated that those changes were not centrally mediated through the GH–IGF-I axis.

Increased IGF-I and decreased IGFBP-2 have been observed in adult volunteers treated with dexamethasone [25]. IGF-I (and IGFBP-3) is positively and IGFBP-2 negatively related to GH secretion [26], suggesting that dexamethasone may augment GH secretion, although direct effects on IGF-I and IGFBP-2 are also possible. In the present study, dexamethasone was administered for 2 weeks to those patients randomised to receive third intensification, beginning at week 35 of chemotherapy. This resulted in suppression of bone alkaline phosphatase and all collagen markers and coincided with variably reduced IGFBP-2 levels, variably increased IGF-I levels but little change in IGFBP-3. We cannot draw definite conclusions as to whether our patients were in a GH resistant state, since formal dynamic function tests of GH secretion would have been inappropriate and urinary GH excretion may be influenced by concomitant drug treatment. However, the lack of suppression of IGF-I and IGFBP-3 at a time when all collagen markers were markedly suppressed suggests that the effects of dexamethasone on the collagen markers and bone alkaline phosphatase were largely through direct effects on target tissue, mediated by local changes in IGF-I via autocrine and paracrine mechanisms [27]. The arrest of lower leg growth observed in our patients during the first 2 weeks was also likely to be due to a direct effect of dexamethasone on the growth plate, as has been demonstrated *in vivo* in rabbits [28] and *in vitro* in rat tibial growth plate chondrocytes [29], mediated through changes in local IGF-I production in the proliferative zone [30].

Because the UKALL XI treatment protocols continue for 2 years, an ALL control group receiving no treatment over the period studied was not available. However, we compared the effects of third intensification (group 2) with those of continuing chemotherapy only (group 1) on bone and soft tissue turnover. For ethical reasons (see Patients and Methods), week 37 measurements were not available for group 1, but there was no significant difference between measurements in weeks 35 and 39 for any of the markers in group 1, suggesting that the short (5 day) course of prednisolone during week 35 had no major lasting effect on bone and soft tissue turnover.

After dexamethasone was stopped in group 2, all markers returned to predexamethasone levels, although bone alkaline phosphatase increased slightly more slowly than the collagen markers and remained low compared with group 1 at week 39. This was similar to our earlier study, when recovery of bone alkaline phosphatase after prednisolone treatment was delayed compared with PICP [13] because of its later synthesis in the osteoblast cycle. No further suppression of any of the markers was observed during thioguanine, cytarabine and cyclophosphamide treatment, no further increase in the markers occurred following completion of third intensification and no difference was observed compared with group 1 after week 39, suggesting that the later phase of third intensification had no major deleterious effect on growth or bone turnover. However, the lack of 'catch-up' in the markers above predexamethasone levels may imply that both the latter part of third intensification and continuing chemotherapy were preventing full poststeroid recovery of bone and soft tissue turnover.

We previously reported that children treated with high-dose i.v. methotrexate as CNS-directed therapy had reduced PICP and bone alkaline phosphatase and increased ICTP and P3NP, compared with those not so treated, suggestive of reduced bone formation, increased bone resorption and subclinical hepatic fibrosis [13]. There was a suggestion in the present study that some of these effects may have persisted through third intensification and continuing chemotherapy to the end of the first year of treatment, although numbers were too small to achieve statistical significance. Bone alkaline phosphatase remained well below the population mean throughout the first year of chemotherapy in the great majority of children, suggesting that bone mineralisation continued to be suboptimal. Although PICP was above the population mean, both before third intensification and at the end of the first year of treatment, it is a less bone-specific marker than bone alkaline phosphatase since (a) a considerable proportion of newly synthesised type I collagen may be degraded without being laid down into bone and (b) type I collagen synthesis occurs in soft tissue (e.g. liver) as well as in bone. Since continuing chemotherapy involved weekly treatment with oral methotrexate, the latter may have contributed

to continuing suboptimal bone mineralisation, as indicated by bone alkaline phosphatase.

In summary, children with ALL randomised to a third intensification block at week 35 of chemotherapy experienced a 2 week decrease in bone and soft tissue turnover during dexamethasone treatment, associated with arrest of lower leg growth. After dexamethasone was stopped, markers returned to pre-intensification levels, but there was evidence of continuing suboptimal bone mineralisation, possibly associated with oral methotrexate treatment. The third intensification phase appears to have added 5–8% to 5-year event-free survival and has (after completion of this study) been incorporated into U.K. protocols as standard treatment (O.B. Eden, University of Manchester, U.K.). The clinical importance of our observations on bone turnover must, therefore, be weighed against improved survival. Longer term follow-up studies are warranted.

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