Evaluation of a New Commercial IRMA for Bone-Specific Alkaline Phosphatase During Treatment with Hormone Replacement Therapy and Calcitonin, Kirsten Overgaard, Peter Alexandersen, Bente Juel Riss, and Claus Christiansen* (Center for Clin. & Basic Res., Ballerup Byvej 222, 2750 Ballerup, Denmark; *author for correspondence: fax +45 4468 4220)

Postmenopausal osteoporosis can be prevented by use of antiresorptive agents, but how the individual response to therapy should be monitored has not yet been settled [1]. Because the concentrations of markers of both bone resorption and formation are 50–100% greater in postmenopausal women than in premenopausal women, and decrease by the same magnitude after a few months of antiresorptive therapy [2, 3], biochemical markers of bone turnover have been suggested as an alternative tool for monitoring the therapeutic response [1, 4, 5].

Recently, a direct IRMA for human bone alkaline phosphatase (B-AP; EC 3.1.3.1) has been developed (Tandem-R Ostease; Hybritech, San Diego, CA). The intraassay variation of this assay is 4–7%, the interassay variation 7–8%, and the detection limit 2.0 μg/L [6]. We used this new IRMA to assay human B-AP in premenopausal (n = 71), early (n = 122), and late (n = 172) postmenopausal women. Furthermore, we investigated the response in early postmenopausal women who completed two 2-year placebo-controlled trials of the effect of different hormone replacement treatment (HRT) regimens. Half of the women participated in study A, in which 20 received estradiol valerate (E2V) and cyproterone acetate (CPA) daily, 21 received sequential E2V and levonorgestrel (LNG; Schering, Berlin, Germany), and 24 received placebo. The other half of the women participated in study B, in which 18 received sequential E2V and medroxyprogesterone acetate (MPA; Organon, Oss, The Netherlands), 20 received sequential estrogen (E2) and desogestrel (DG; Organon), and 19 received placebo. Dosage details of studies A and B are given in the legend to Fig. 1. Finally, we investigated women of ages 68–72 years, who were randomized to daily treatment with salmon calcitonin (SCT; SANDOZ, Basel, Switzerland)—50 IU (n = 39), 100 IU (n = 42), 200 IU (n = 40)—or placebo (n = 40). Informed consent was obtained from all participants according to Helsinki Declaration II, and the trials were approved by the Ethical Committee of Copenhagen County.

Bone mineral content of the individuals in studies A and B was measured in the distal forearm by single-photon absorptiometry every 3 months during the 2-year period of the studies. Bone mineral content of the lumbar spine was measured by dual-energy absorptiometry every year and its bone density every 3 months.

Treatment groups were well matched with placebo groups in the study populations (data not shown). In the 50- and 70-year-old women, the mean values of B-AP were 146% and 143% of the mean in premenopausal women, respectively. Z-scores (the difference from the values in premenopausal women, expressed in standard deviations) were respectively 1.3 and 1.2 for the 50- and 70-year-old women. Fig. 1 shows the mean values (+SE) of B-AP in study groups A and B during treatment with HRT. Gradual decreases of 40% were seen in all four HRT groups, reaching a minimum within 12 months (P < 0.001) and remaining there during the rest of the study. There were no statistically significant differences in the decline in B-AP (calculated as the slopes) of the four HRT groups. The placebo groups showed virtually no changes. Correlating the 12-month values of B-AP to the bone loss over 2 years in the 50-year-old women on HRT showed that the B-AP concentrations were significantly negatively correlated with the bone mass changes in the distal forearm (r = 0.62) and the spine (r = 0.62).

Intranasal treatment with SCT decreased B-AP more than placebo did (P < 0.05), but there was no dose–response effect. B-AP in the placebo (calcium only) group also decreased but by significantly less than in the actively treated groups (P < 0.05).

B-AP is localized in the plasma membrane of the osteoblast and is released into the circulation as a dimer. The exact role of B-AP in unknown, but in vivo it may be involved in bone formation and skeletal mineralization [7]. Using the present assay, investigators have consistently demonstrated [6, 8, 9] a cross-reactivity with the liver component of total AP of 11–16%. Given this 16% cross-reactivity and the assumption that, in nongenetic diseases such as liver disease, an increase in total AP would be accounted for only by an increase in liver AP, the present B-AP assay can be used validly as long as the total AP does not exceed 2.6 times the upper limit of the normal range [6]. Accordingly, Garnero and Delmas showed that determinations of B-AP fell within the normal range in patients with liver diseases whose total AP activity was <4.5 μkat/L [6]. However, serum samples from patients with more severe liver disease and much greater increases in total AP may of course also show high results for B-AP.

We found a gradual decrease in B-AP in response to four different HRTs. As a marker of bone formation, this relatively slow decrease was expected. The decrease was comparable with the decrease in B-AP after bisphosphonate treatment of elderly

**Fig. 1.** Serial mean ± SE values of B-AP during 2 years of four different estrogen/progestogen treatment regimens.

In study A (upper panel), 20 women received 2 mg of E2V and 1 mg of CPA every 2 days (□). 21 received sequential E2V and LNG (days 1–16: 2 mg of E2V; days 17–28: 2 mg of E2V and 75 μg of LNG) (■), and 24 received placebo (○). In study B (lower panel), 18 women received sequential E2V and MPA (days 1–11: 2 mg of E2V; days 12–21: 2 mg of E2V and 10 mg of MPA; days 22–28: no treatment) (△), 20 received sequential E2V and DG (days 1–12: 1.5 mg of E2; days 13–24: 1.5 mg of E2 and 150 μg of DG; days 25–28: no treatment) (▲), and 19 received placebo (○).

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osteoporotic women [10]. The relative decrease (40%) was of the same order as the decrease in osteocalcin seen in such situations but was more pronounced than the decrease in total AP [11].

After only 3 months of HRT, the changes in B-AP correlated significantly with the 2-year changes in bone mass. Because of the gradual decrease in B-AP, the optimal correlation was reached after 12 months, where a new steady state was reached. The correlation coefficients were of the same magnitude as seen for other markers [4].

We have elsewhere found (unpublished manuscript, submitted for publication) that a new marker of bone resorption (CrossLaps™, a degradation product of type I collagen) responds in a dose-dependent way to treatment with nasal SCT. B-AP, however, did not show any dose dependency in response to SCT therapy.

The present sensitivity of B-AP for the changes at menopause was in accordance with the findings in a previous study by our group [11]. Another group, however, applying the same method, found a greater sensitivity of B-AP to the menopausal changes [6]. We have no explanation for the discrepancy; the study populations of all three publications were recruited as being healthy without diseases interfering with bone metabolism.

In conclusion, our evaluation of B-AP as determined with a newly developed IRMA shows that this marker is highly responsive to the changes in calcium metabolism observed after menopause. In particular, B-AP decreases significantly when HRT (and SCT) is instituted. The change from baseline reflects the effect of the therapy as soon as a new steady state has been reached. The absolute value of B-AP also correlates with the rate of bone loss.

References


A major consideration in interpretation of digoxin serum values is interferences by endogenous digoxin-like immunoreactive factors (DLIF), which may cause falsely high values to be reported in patients receiving digoxin treatment, particularly in newborns, women in the second or third trimester of pregnancy, and patients with renal failure or liver disease [1]. DLIF interference is more consistently found in newborns, in whom DLIF may reach apparent digoxin concentrations within the therapeutic range in some patients not receiving digoxin [2–5]. DLIF interference is observed in most digoxin immunoassays [6, 7], including the TDx FPIA Digoxin assay [3] and the new TDx FPIA Digoxin II assay [4, 5], both from Abbott Labs., Abbott Park, IL. New digoxin immunoassays try to reduce DLIF interferences by two mechanisms: pretreating clinical samples to diminish or even eliminate DLIF, and using more-specific antibodies with less cross-reactivity with DLIF [8].

Evaluation of DLIF interference in the new digoxin immunoassays is important, particularly for laboratories that serve neonatal patients. Here, we compared the interference of DLIF in the Abbott TDx FPIA Digoxin II, one of the most widely used immunoassays, with that in two newly marketed digoxin immunoassays, the Svy Em 2000 Digoxin Assay (Syva, Palo Alto, CA) and the Roche Digoxin “On Line” (Roche Diagnostics, Nutley, NJ), in samples from newborns and pregnant women.

We carried out this prospective study from January to March 1995, using specimens with no digoxin present: 20 umbilical cord blood samples from healthy newborns, and 20 samples from women in the third trimester of pregnancy. The pregnant women were not the mothers of any of the babies studied. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Whole blood (5 mL) was collected without any anticoagulant from pregnant women by peripheral venipuncture immediately before delivery. Newborns’ samples were obtained from umbilical cord immediately after delivery. After centrifugation, serum was separated into three aliquots and frozen at −70 °C until assay (no more than 8 weeks from sampling). All samples, both from newborns and pregnant women, were assayed together by each method in the same day. Clinical records and hospital treatment sheets were reviewed to confirm that the patients had no other pathology and were not currently or previously being treated with cardiac glycosides.

Digoxin concentrations were measured by a TDx analyzer with the TDx Digoxin II reagent pack, by a Roche (Branchburg, NJ) Cobas Mira Plus analyzer with the Digoxin On Line kit, and by the Syva Em 2000 Digoxin Assay, also performed on a Cobas Mira analyzer. Kits were used according to the manufacturers’ instructions. Low-, medium-, and high-concentration controls with 0.8, 2.6 and 3.2 μg/L digoxin (Dade TDM Control; Baxter Diagnostics, Deerfield, IL) were included in each assay—all of which showed intraassay CVs <10%.