Is Bone Alkaline Phosphatase an Adequate Marker of Bone Metabolism During Acute Corticosteroid Treatment? Anne Petrez,1,2 Muriel Moris,1 Dominique Willem,1 and Pierre Bergmann1 (1 Rheumatol. Clin., Dept. of Intern. Med., 2 Lab. of Clin. Chem. and Exp. Med., Brugmann Univ. Hosp., Place Arthur Van Gehuchten 4, B-1020 Bruxelles, Belgium; *author for correspondence: fax 32 2 477 21 78, e-mail pbergman@resulb.ulb.ac.be)

Corticosteroids are known to decrease bone formation. This effect is evidenced by a decrease in the serum concentrations of biochemical markers of bone formation such as serum osteocalcin (BGP) or type I procollagen propeptide (PICP) [1, 2]. Cross-sectional studies have shown differences in the magnitude and time course of the response, depending on the investigated serum bone marker, the corticosteroid dose, and the route of administration [3]. High doses of intravenous methylprednisolone (pulse MPS) have been recently shown to induce a rapid and important decrease in BGP [4] and PICP [5]. Recently, the measurement of bone alkaline phosphatase (BAP) by IRMA was proposed as a new marker of osteoblastic function. BAP having been shown to be quite discriminant in several conditions of increased bone turnover, e.g., postmenopausal osteoporosis [6]. In this longitudinal study, we report the evolution of BAP, BGP, and PICP during the first 72 h after MPS administration.

Seven patients with severe rheumatic diseases (rheumatoid arthritis, polymyositis, and Reiter disease) received a course of pulse MPS, pulse therapy having been indicated for a flare of the disease. None of the patients had a severe underlying disease such as diabetes, cirrhosis, neoplasia, cardiac insufficiency, or metabolic bone disease, and they had satisfactory renal function (serum creatinine <15 mg/L). One patient was studied during two pulses. Oral informed consent was obtained from each patient.

The patients were admitted to the hospital 36 h before the first pulse (day 0). The following day (day 0, baseline assessment), a venous catheter was placed in the forearm and an infusion of 50 g/L glucose in water was given (1 L/day). Blood samples were drawn at 0, 3, 6, 12, and 24 h for baseline assessment. The next day (day 1), 1 g of MPS was given in isotonic saline over 30 min, and blood was sampled at 3, 6, 12, 24, 48, and 72 h afterwards. In each patient, sampling was initiated at 0900 to minimize the effects of circadian rhythm. For RIA measurements, blood was centrifuged without delay at 4°C after clotting, and sera were kept frozen at −20°C for no longer than 6 months until analyzed. To eliminate interassay variation, we measured all samples from each patient in the same assay.

BGP and PICP were measured by RIA (with assays from Incstar, Stillwater, MN, and Farmos Diagnostica, Turku, Finland, respectively). BAP was measured by an IRMA in which the two monoclonal antibodies used were directed against unique epitopes of the skeletal AP molecule (Tandem-R Ostase; Hybritech Europe, Liège, Belgium). According to the manufacturers, the minimum detectable concentration was 0.2 μg/L for BGP, 1.2 μg/L for PICP, 2 μg/L for BAP. The intraassay CV was 6% for BGP, 3% for PICP [7], and 7% (manufacturer’s data) for BAP. Results were expressed as mean ± SE. For each treatment, the evolution of the bone markers with time (expressed as a percentage of the initial value) was analyzed by one-way ANOVA for repeated measurements. Multiple comparisons were made by using the Tukey test. Differences between bone markers were identified by two-tailed t-test, after ANOVA analysis.

During baseline assessment, the bone markers were within their respective reference ranges (1.6–4.8 μg/L for BGP, 55–165 μg/L for PICP, and 3.6–21.2 μg/L for BAP) in all of the patients except for two patients in whom BGP was 1.3 and 0.8 μg/L. No substantial variations in BGP, PICP, and BAP were observed during baseline assessment, although a trend to decrease toward a minimum at 1500 (0 time + 6 h) was observed for BGP and PICP (Fig. 1). After the pulse, BGP decreased significantly (baseline: 1.7 ± 0.5 μg/L, nadir: 0.7 ± 0.1 μg/L, P <0.001) in each patient, including the two with low baseline.

Fig. 1. Evolution of osteocalcin (BGP), type I collagen propeptide (PICP) and bone alkaline phosphatase (BAP) during baseline assessment (not significant) and after intravenous administration of 1 g of methylprednisolone (MPS). Results are expressed as mean ± SE. BGP and PICP evolution with time: significant (Tukey test; P <0.001) vs time at 0 and 3 h (+); vs time at 12, 24, and 48 h (+).
MPS. BGP began to decrease significantly as early as 6 h after the pulse. The nadir was reached 24 h after the pulse, and a return to initial values was observed by 72 h (Fig. 1A). The evolution of PICP paralleled that of BGP (baseline: 93 ± 8 μg/L, nadir: 55 ± 5 μg/L, P <0.001) (Fig. 1B). However, the PICP decrease was slower and significantly different from that of BGP at the time of the nadir (59% ± 1% of baseline value vs 41% ± 1%; P <0.05). The serum BAP concentration over this time did not vary significantly from its baseline value, 9.7 ± 1.6 μg/L.

The present study confirms that high intravenous doses of MPS given as a short infusion (pulse) dramatically and rapidly reduce BGP, as shown recently by Cosman et al. [4]. Moreover, we have demonstrated that the evolution of PICP is parallel to that of BGP. PICP, however, was less sensitive to the inhibitory effect of MPS, in agreement with previous data in corticosteroid-treated patients and in metabolic bone diseases [8]. The reason for the relative discrepancy between these two markers is at present unclear and might depend on the assay. Ebeling et al. [8] have suggested that the PICP RIA recognizes epitopes different from those on the native PICP molecule. The design of our study allowed us to show that the inhibitory effect of MPS pulses on BGP and PICP was transient and short. In contrast with the evolution of BGP and PICP, BAP concentrations did not decrease significantly after the MPS pulse. This recently developed IRMA, which uses two monoclonal antibodies that react preferentially with BAP and exhibits low cross-reactivity with liver AP, has been a valuable and sensitive marker in assessing osteoporosis and metabolic bone disorder [9]. Few data have been published on the amounts of BAP in patients receiving corticosteroids, although BAP values have been reported in a study using the lectin-presentation technique [10]. In that study, BAP was less sensitive to corticosteroids than BGP.

The physiological basis of this discrepancy between BAP and the other markers could be that short-term exposure of osteoblast-like cells to corticosteroids induces the mRNA coding for alkaline phosphatase while it decreases the mRNAs of BGP [11] and collagen [12]. A longer half-life of AP in the circulation than for BGP and PICP might also help explain the discrepancy between the markers [13]. Also, BGP and PICP are both structural proteins without enzymatic activity—in contrast to BAP, an enzyme without known structural properties. Unfortunately, we did not measure the enzymatic AP activity in our study, and so cannot exclude the possibility that the enzymatic activity of BAP is affected by corticosteroid while the protein mass remains unchanged. In a population of osteoporotic patients, however, Farley et al. [14] have shown that the enzyme measurements were highly correlated with the immunoassay.

In conclusion, BAP (measured by an IRMA) appears less sensitive than BGP and PICP to the inhibitory effects of MPS, at least during acute treatment. On the other hand, this comparison of BAP with BGP or PICP shows that these measurements, which reflect different osteoblastic functions, can diverge in some situations. Sometimes, therefore, the determination of both structural and enzymatic markers could be more informative than either one alone.

References

14. Farley JR, Hall SL, Flacca D, Orecut C, Miller BE, Hill CS, Baylink DJ. Quantification of skeletal alkaline phosphatase in osteoporotic serum by wheat germ agglutinin precipitation, heat inactivation, and a two-site immuno-

Simple, Rapid Nonradioactive Method to Detect Major Cystic Fibrosis Mutations in Ashkenazi Jews. Boaz Avidor,1 Haim Zakut, and Batshva Kerem2 1 Dept. of Obstet. and Gynecol., Sackler Faculty of Med., Tel Aviv Univ., The Edith Wolfson Med. Center, Holon 58100, Israel; 2 Dept. of Genetics, Life Sci. Inst., Hebrew Univ., Jerusalem 91904, Israel; *address for correspondence: The Bernard Fridan Lab. for Molec. Biol. and Infect. Dis., Tel Aviv—Elia Sourasky Med. Center, Ichilov Hosp., 6 Weizman St., Tel Aviv, Israel 64239; fax 972-3-697-3850

Cystic fibrosis (CF), a common lethal recessive disorder in the Caucasian population, affects 1 in 2500 live births and has a high carrier frequency (1 in 25) [1]. A major mutation causing the disease, ΔF508, has been found in 70% of the CF chromosomes around the world [2]. More than 500 additional mutations have been described (Cystic Fibrosis Genetic Analysis Consortium 1994; personal communication).

The Ashkenazi Jews are one of the few ethnic groups having a predominant CF mutation other than ΔF508. Previous studies revealed a major mutation, W1282X, which accounts for 50% of the CF chromosomes in this ethnic group in Israel and North America [3–5]. If one can detect this mutation, the ΔF508 mutation (23%), and three other mutations (3849+10 kb C→T, N1303K, G542X), one can identify 95% of the CF-causing mutations in this patient population [4, 5]. This very high