Bone Alkaline Phosphatase (BAP)

A biochemical marker of bone turnover

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Summary

The increasing number of drugs available for treatment of bone diseases requires the use of more rapid and predictive methods to assess therapy efficacy. While detectable and significant changes in bone mineral density (BMD) take 18 to 24 months to develop, bone turnover marker have been shown to detect changes in bone tissue within 3-6 months after starting anti-resorptive therapy. Therefore, measurement of bone turnover marker is increasingly recommended as a key component of therapy management: to rapidly identify therapy responders and non-responders, to assess therapy efficacy and to determine the optimal therapy and dose of treatment. Moreover, since biochemical bone marker reflect the whole-body rates of bone turnover, the combined measurement of bone marker and BMD provides more information on overall bone loss than BMD measurement at specific skeletal sites alone.

This paper presents an overview of all relevant clinical and technical data on Bone-Specific Alkaline Phosphatase (BAP), a biochemical marker of bone formation. Seven key criteria were formulated that need to be fulfilled by a biochemical marker to be useful in assessing bone turnover and monitoring therapy. Using these criteria, BAP was compared to other marker, whereby BAP demonstrated to be one of the most attractive bone turnover marker to date.

Important technical aspects as cross-reaction with liver alkaline phosphatase and BAP measurement in units of activity (U/L) vs. units of mass (μg/L) are extensively discussed.

Summaries of the most important clinical studies with BAP as bone-turnover marker are presented. All clinical data have been obtained with the Quidel® BAP Assay. Clinical study conclusions are:

Increased serum levels of BAP are seen in conditions characterized by excessive bone turnover including postmenopausal women, osteoporosis, Paget’s disease, hyperparathyroidism, thyrotoxicosis, and metastatic cancer, and are associated with rapid bone loss. BAP levels decrease following anti-resorptive therapy in a dose-dependent manner. These short-term changes are inversely correlated with long-term changes in BMD. BAP levels are correlated with bone growth in children and reflect pubertal growth stages.

In summary, it is demonstrated that BAP identifies rapid bone losers, and accurately monitors the efficacy of hormone replacement-, bisphosphonate-, PTH analogue- and growth hormone-therapies.
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1 Introduction

1.1 Osteoporosis and bone remodeling
Bone remodeling is an ongoing dynamic process consisting of bone resorption (due to osteoclasts digesting type I collagen) and bone formation (due to osteoblasts). Normally, these processes are balanced, resulting in 10% replacement of the skeleton, each year. However, due to aging, disease or other conditions, bone turnover may become imbalanced where bone resorption and formation occur at different rates. Osteoporosis is an age-related bone disease characterized by low bone mass and micro architectural deterioration of bone tissue (see Figure 1). It is diagnosed often after an already unacceptable loss of bone has occurred. In many cases a fracture leads to the initial diagnosis.

Fig. 1
Pictures provided by Pr. Daniel Chappard Université d’Angers, France

1.2 Medical conditions affecting bone remodeling
Osteoporosis is the main clinical condition affecting bone remodeling, afflicting an estimated one-third of women aged 60–70, and two-thirds of women aged 80 or older. Osteoporosis can be prevented with proper diet, exercise, and elimination of controllable risk factors; it can be treated with anti-resorptive therapies.

Metabolic bone disorders include:
- Hyperparathyroidism
- Hyperthyroidism
- Paget’s disease – a condition of abnormal bone formation
- Metastatic cancer to bone
- Nutritional rickets and osteomalacia
- Multiple myeloma
- Malabsorption syndrome
- Disorders caused by drug therapies:
  - immunosuppressive drugs for treating cancer and organ transplants
  - heparin, used in kidney dialysis
  - phenytoin (Dilantin®) for epilepsy
  - glucocorticoids (corticosteroids) for rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and asthma
  - aluminium-containing antacids
### 1.3 Drug therapies for metabolic bone diseases

Physicians routinely treat patients with metabolic bone diseases. An overview of current and promising new therapies are listed in Table 1.

#### Table 1. Therapies for treatment of metabolic bone disease.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-resorptive agents:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen (hormone replacement)</td>
<td>Because of side effects, for transitory time of peri-menopause only.</td>
<td>1</td>
</tr>
<tr>
<td>Phytoestrogen (hormone replacement)</td>
<td>Natural source of estrogen as found in soy. Used by women as a “safe” alternative to HRT.</td>
<td>2</td>
</tr>
<tr>
<td>Calcium</td>
<td>Prevention of osteoporosis.</td>
<td>3, 4</td>
</tr>
<tr>
<td>Selective estrogen receptor modulators</td>
<td>Prevent bone loss and the risk of vertebral fracture (Raloxifene, (Evista))</td>
<td></td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td>Prevent bone loss and increase BMD (Alendronate (Fosamax); Risedronate (Actonel)). Rigid administration is a disadvantage.</td>
<td>5</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Treatment of osteoporosis and Paget’s disease, considered not as effective as bisphosphonates. Decreased tolerance with long-term use.</td>
<td>6, 7</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Active form of vitamin D given to post-menopausal women who have osteoporosis in the spine.</td>
<td>8, 9</td>
</tr>
</tbody>
</table>

**Formation stimulating agents:**

- Sodium fluoride: Increases BMD, however, clinical studies showed no decrease in vertebral fracture rates

- Parathyroid hormone (human recombinant PTH (1-34)): PTH initially stimulates bone formation and later increases bone remodeling; increases spinal BMD. Suggested for treatment of patients with persistent osteoporosis after prior alendronate treatment. (Teriparatide (Forteo))

- Growth factors: Growth hormone therapy is used (and FDA approved) in the treatment of hypo-pituitarism and somatotropin deficiency of children and adults.

**Agents inhibiting resorption and stimulating formation:**

- Strontium ranelate: Inhibits bone resorption and stimulates bone formation.

References:

1. [Reference](#)
2. [Reference](#)
3. [Reference](#)
4. [Reference](#)
5. [Reference](#)
6. [Reference](#)
7. [Reference](#)
8. [Reference](#)
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10. [Reference](#)
11. [Reference](#)
12. [Reference](#)
13. [Reference](#)
14. [Reference](#)
15. [Reference](#)
2 Diagnostic methods for detecting metabolic bone diseases

2.1 Diagnosis of metabolic bone disease, Bone Mineral Density (BMD)

Diagnosis of metabolic bone diseases can be established by measuring bone mass, i.e. bone mineral density (BMD) at the hip, spine or other location. However, BMD is a static measure of bone composition, reflecting its history. A baseline BMD value does not offer any prediction of future bone loss or response to therapy. Moreover, since biochemical bone marker reflect the whole-body rates of bone turnover, the combined measurement of bone marker and BMD provides more information on the overall bone loss than BMD measurement at specific skeletal sites alone. Finally, while BMD can indicate bone loss, it does not provide information on alteration or deterioration of bone tissue structure.

The increasing number of drugs available for treatment of osteoporosis and other bone diseases requires the use of more rapid and predictive methods to assess therapy efficacy. While detectable and significant changes in BMD take 18 to 24 months to develop, bone turnover marker have been shown to detect changes in bone tissue within 3-6 months after starting anti-resorptive therapy. Therefore, measurement of bone turnover marker is increasingly recommended as a key component of therapy management: to rapidly identify therapy responders and non-responders, to assess therapy efficacy and to determine the optimal therapy and dose of treatment.

2.2 Bone marker for detecting bone disease and assessing therapy efficacy

To be useful in assessing the rate of bone turnover, and monitoring therapy, marker should:

A. show a difference in the rate of bone turnover pre-and post-menopause
B. demonstrate minimal analytical variation
C. significantly change in response to treatment
D. detect change in short time interval (months)
E. demonstrate minimal within person (biological) variation
F. preferably demonstrate little variation over the day
G. preferably demonstrate no influence to food intake
H. preferably demonstrate high stability in the biological specimen

An overview of current and promising new tests is shown in Table 2, together with the score on the criteria mentioned above (adapted from Caulfield et al. [14]).

Based on these criteria, BAP (Bone Specific Alkaline Phosphatase) appears to be one of the most attractive bone turnover marker.
Table 2. Bone marker and characteristics.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Method</th>
<th>Main Sample Type</th>
<th>Pre- &amp; post-menopause</th>
<th>Analytical variation</th>
<th>Treatment response</th>
<th>Short time change</th>
<th>Within person variation</th>
<th>Daily variation</th>
<th>Food intake</th>
<th>Sample stability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAP</td>
<td>ELISA</td>
<td>Serum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5 days 2–8 °C</td>
</tr>
<tr>
<td>Osteocalcin Intact</td>
<td>IRMA/ELISA</td>
<td>Serum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>4 hours 2–8 °C</td>
</tr>
<tr>
<td>Osteocalcin N-mid</td>
<td>ELISA</td>
<td>Serum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5 days 2–8 °C</td>
</tr>
<tr>
<td>PINP</td>
<td>RIA</td>
<td>Serum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5 days 2–8 °C</td>
</tr>
<tr>
<td>PICP = CICP</td>
<td>ELISA</td>
<td>Serum</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>5 days 2–8 °C</td>
</tr>
<tr>
<td><strong>Resorption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAP 5b</td>
<td>ELISA</td>
<td>Serum</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>NA</td>
<td>+</td>
<td>±</td>
<td>2 days 2–8 °C</td>
</tr>
<tr>
<td>DPD</td>
<td>ELISA</td>
<td>Urine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>7 days 2–8 °C</td>
</tr>
<tr>
<td>NTx</td>
<td>ELISA</td>
<td>Serum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>1 day 2–8 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>3 days 2–8 °C</td>
</tr>
<tr>
<td>CTx</td>
<td>ELISA</td>
<td>Serum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>1 day 2–8 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7 days 2–8 °C</td>
</tr>
<tr>
<td>ICTP = CTX-MMP</td>
<td>ELISA</td>
<td>Serum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>±</td>
<td>+</td>
<td>5 days 2–8 °C</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sRANKL</td>
<td>ELISA</td>
<td>Plasma</td>
<td>NA</td>
<td>+</td>
<td>±(b)</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1 day 2–8 °C</td>
</tr>
<tr>
<td>Osteoprotegerin (OPG)</td>
<td>ELISA</td>
<td>Plasma</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1 day 2–8 °C</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>ELISA</td>
<td>Serum</td>
<td>NA(c)</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2 days 2–8 °C</td>
</tr>
</tbody>
</table>

Scores are: + (yes), - (no), ± (fair/indeterminate), NA (not available)

(a) Letters correspond to following, the marker:
A. shows a difference in the rate of bone turnover pre-and post-menopause
B. demonstrates minimal analytical variation
C. significantly changes in response to treatment
D. detect changes in short time interval (months)
E. demonstrates minimal within person (biological) variation
F. preferably demonstrates little variation over the day
G. preferably demonstrates no influence to food intake
H. preferably demonstrates high stability in the biological specimen
(b) In contrast to OPG, sRANKL showed no response to bisphosphonate treatment of osteoporotic postmenopausal women [43]. However, PTH treatment of glucocorticoid induced osteoporotic women resulted in a fast (1 month) and sustained increase of sRANKL levels [44].
(c) Cathepsin K is elevated in patients with established rheumatoid arthritis.
3 BAP as a marker for detecting changes in bone turnover

3.1 BAP background

Bone-specific alkaline phosphatase (BAP) is synthesized by the osteoblasts and is presumed to be involved in the calcification of bone matrix, though its precise role in the formation process is unknown. BAP is one of a number of different isoenzymes of alkaline phosphatase: bone, liver, kidney, intestine, and placenta. In the serum of most healthy individuals, bone and liver isoenzymes of the tissue non-specific AP gene predominate in approximately equal proportions. The difference in glycosylation of the bone and liver isoenzymes (products of the same gene) has been exploited to generate specific antibodies against BAP. BAP is considered to be a highly specific marker of the bone-forming activity of osteoblasts.

3.2 Methods for measuring alkaline phosphatase

Diagnostic tests that are used for detecting alkaline phosphatase are:

- **BAP ELISA** – routine enzyme-linked immunosorbent assay, measuring BAP enzyme activity.
- **BAP IRMA** – routine immunoradiometric assay measuring BAP in protein mass units.
- **Total alkaline Phosphatase (TAP)** – routine automated laboratory test. Its high cross-reactivity to liver alkaline phosphatase makes this method non-specific for diagnosing bone disease.
- **Electrophoresis** – different in-house methods available. Non-standardized, results are inconsistent from lab-to-lab.
- **Lectin Precipitation** – different in-house methods, inconsistent from lab-to-lab.

Correlations between various methods are shown in Figure 2.

Fig. 2. Correlation between BAP ELISA (Quidel®) and other methods for determining BAP.
3.3 Measurement of BAP in protein mass (μg/L) and enzyme activity (U/L)

Bone Alkaline Phosphatase (BAP) is a marker for osteoblastic activity in vitro. BAP is typically measured by one of the two methods, protein mass or enzyme activity.

**Enzyme Activity**

BAP is bound to a monoclonal antibody specific for the bone isoform. Other forms of Alkaline Phosphatase (e.g., liver, etc) are washed away. Alkaline Phosphatase activity is measured via a chromogenic (color change), chemical reaction. In the Quidel® BAP Assay, results are determined directly from this color change and expressed as units per liter (U/L). As the enzyme activity is measured the preferred expression is U/L however, the enzyme activity can also be calculated in μg/L.

**Protein Mass**

In the IRMA method, the enzyme molecule BAP is directly measured by using two monoclonal antibodies (Sandwich Assay) detecting two different epitopes; results are expressed in mass units (μg/L).

**Correlation Protein Mass and Enzyme activity expressed in μg/L**

BAP measured with the protein mass and the enzyme activity method, both expressed in μg/L, showed an excellent correlation.
3.4 BAP cross-reaction with liver alkaline phosphatase

As outlined above, all BAP assays work by passing Total Alkaline Phosphatase activity "through a sieve", whereby the bone isoform (BAP) is retained by the monoclonal antibody. Therefore, the specificity of the monoclonal antibody for BAP determines the assay's cross-reactivity with liver alkaline phosphatase (AP). In subjects with high liver AP (if the liver, bile ducts or gallbladder system are not functioning properly, e.g. alcoholic liver disease, liver carcinoma), the results of BAP measurements may be artificially high. In case of concomitant bone disease, artificially high BAP values may obscure the patient's response to anti-resorptive therapy.

In a study by Sokoll [45], BAP values as generated by the Quidel® BAP assay and a BAP IRMA in subjects with liver and bone disease were examined. They noted that the number of liver disease subjects with values increased above the upper limit of the healthy reference range was greater for the IRMA than for Quidel® BAP. Of all assays, the Quidel® BAP assay has the lowest cross reactivity with liver AP only 6 to 8 %.

3.5 Daily, dietary and age-related variation of BAP.

BAP shows no significant circadian variation in serum as reported by many studies (for example, see Tobiume et al. [37]). In contrast to Total Alkaline Phosphatase, BAP levels are in general unaffected by diet [41, 42]. Age-related variation of BAP and other bonemarker in women is shown in figure 3. Values significantly increase during menopause (Iki et al. [38]).

Fig. 3. Bone turnover marker values as a function of age

Total serum alkaline phosphatase levels decreased significantly during LCHP diets from the usual diet, but bone-specific alkaline phosphatase levels did not differ.
4 BAP clinical data

All clinical data shown are obtained with the Quidel® BAP Assay.

4.1 Summary

Increased serum levels of BAP are seen in conditions characterized by excessive bone turnover including postmenopausal women, osteoporosis, Paget’s disease, hyperparathyroidism, thyrotoxicosis, and metastatic cancer, and are associated with rapid bone loss [16]. BAP levels decrease following anti-resorptive therapy in a dose-dependent manner. These short-term changes are inversely correlated with long-term changes in bone mineral density. BAP levels are correlated with bone growth in children and reflect pubertal growth stages.

4.2 BAP responses to different clinical conditions and therapy

4.2.1 BAP indicates increased bone turnover

BAP is a sensitive indicator of the increased bone turnover that occurs in postmenopausal women and in osteoporosis. BAP was measured in 107 postmenopausal females (age 44-88) and 191 premenopausal controls. Distribution of the BAP values [28] demonstrates that women in the postmenopausal group showed higher levels of BAP relative to their normal, premenopausal counterparts, indicating higher levels of bone remodeling occurring in this age group versus normal, premenopausal women.
4.2.2 Association between BAP levels and rapid bone loss

A strong association between BAP levels and rapid bone loss was reported from the Hawaii Osteoporosis Study cohort. The graph to the right expresses the data as a probability of rapid bone loss given a BAP level above or below the mean for the cohort. Women with BAP levels 2SD below the mean had a very low probability of rapid loss whereas women with the highest levels of BAP had an 80% probability of losing bone rapidly. The relationship appears to be continuous [16]. The association between BAP and bone loss is equivalent to that between BMD and fracture and suggests the marker can play a valuable role in risk assessment.

4.2.3 BAP reflects antiresorptive effect of HRT in postmenopausal women

In the multicenter Postmenopausal Estrogen/Progestin Interventions trial (PEPI), 875 postmenopausal women (45–64 years) randomly received either placebo or conjugated equine estrogens. This occurred with or without one of three progestin regimens in a double-blind trial of the efficacy hormone replacement therapy (HRT) for preserving or increasing bone mass. Bone mineral density of the lumbar spine at baseline and after 1 and 3 years of therapy increased by up to 5.0% in the HRT groups and decreased 1.8% in the placebo group. Mean BAP levels decreased 32% on average from baseline reaching nadir levels at 12 months in HRT-treated women, a significant difference from the women in the placebo group. Over 4/5ths of the HRT-treated women experienced a BAP decrease of 20% or more and virtually all had values within the reference range after 12 months [17].

To identify therapy responders and non-responders the International Osteoporosis Foundation (IOF) [47] has formulated the following recommendation: for a 90% specificity to predict a positive BMD response (+3%), the BAP value of an individual patient should decrease 20 to 40% from the baseline BAP value.

4.2.4 Change in BAP predicts BMD change in HRT- and placebo-treated women

BAP data from the women in the PEPI trial were divided in quartiles to demonstrate the strong relationship between change in bone turnover and change in lumbar spine bone mineral density (BMD). The women in the first quartile whose BAP decreased the most in one year experienced the greatest increase in BMD. The women in the fourth quartile whose BAP decreased minimally, or actually increased, experienced a decrease in BMD. BAP was also predictive of BMD changes at the hip at 3 years and both spine and BMD changes at 1 year [17].
4.2.5 BAP rapidly identifies more responders to HRT than BMD

The usefulness of a diagnostic test for monitoring the effects of drug therapy depends upon the ability of the test to differentiate between a true biological response and the normal variation that occurs within individuals.

In the PEPI trial, the average long-term intra-individual coefficient of variation (CV) was determined in the placebo group (BAP, 7.8 %; BMD, 1.0 %) and used to determine the number of true biological responders to HRT.

Following 12 months of therapy, test levels had decreased by more than 2 times the long-term variability in 85 % of the women for BAP and 69 % of the women for BMD. BAP thus offers both a greater and earlier ability to detect response to HRT in postmenopausal women [17].

4.2.6 BAP reflects antiresorptive effect of alendronate in osteoporotic women

In a multicenter study conducted by Merck, 994 postmenopausal women with osteoporosis – defined as having a spinal BMD (DEXA) equal to or more than 2.5 SD below that for healthy young women – had been randomized to receive either placebo or one of three doses of alendronate in a double blind trial of the efficacy of alendronate for preserving or increasing bone mass.

BMD of the lumbar spine measured at baseline and following 12 months of therapy increased 5.5 % in the alendronate group and decreased 0.6 % in the placebo group. Serum samples were available for 180 women in the placebo group and 134 in the alendronate 10 mg/day group.

Mean BAP levels decreased 31 % (p < 0.00001 compared to placebo) after 3 months and 43 % after 6 months. 90 % of alendronate-treated women had a BAP decrease of 20 % or more after 6 months.

BAP levels declined slightly from baseline (p < 0.01) in the placebo group, likely as a result of the bone-sparing effect of the 500 mg/day calcium supplement all subjects were receiving [18].
4.2.7 Baseline bone turnover predicts turnover response to alendronate

Measuring bone turnover with a marker such as BAP may determine which patients will respond best to anti-resorptive therapy given to reduce turnover. Since bone turnover is an independent risk factor for fracture, a reduction in turnover is a valid clinical outcome of therapy.

In a multicenter study conducted by Merck, 126 postmenopausal women with osteoporosis received alendronate 10 mg/day in a placebo-controlled, double-blind trial of the efficacy of alendronate for preserving or increasing bone mass. Mean BAP levels decreased steadily from baseline, reaching nadir levels at 6 months. Baseline levels of BAP were highly negatively correlated (r = -0.83) with the change in BAP that had occurred by 6 months.

Women with higher baseline levels of BAP were more likely to experience a decrease in bone turnover than women with lower baseline levels [18].

4.2.8 Response to alendronate is more rapidly identified by BAP than by BMD

The usefulness of a diagnostic test for monitoring the effects of drug therapy depends upon the ability of the test to differentiate between a true biological response and the normal variation that occurs within individuals.

In the alendronate trial, the average long-term intra-individual coefficient of variation (CV) was determined in the placebo group (BAP, 10.0 %; LS BMD, 1.94 %) and used to determine the number of true biological responders to alendronate. Following 6 months of therapy, BAP levels had decreased by more than 2 times the long-term variability in 90 % of the women. 80 % of the women exhibited a lumber spine BMD response at 3 years using the same criteria. BAP thus offers both a greater and earlier ability than BMD to detect response to alendronate in osteoporotic women [18].

4.2.9 BAP is more sensitive than Total AP to alendronate in osteoporosis

BAP offers a more sensitive measure of changes in bone turnover than total alkaline phosphatase activity (total AP) by virtue of its high specificity for bone. This is illustrated in a study of 121 osteoporotic women treated for one year with alendronate 10 mg/day. BAP decreased by over twice as much as total alkaline phosphatase at all time points measured. The differences in the changes of BAP and total AP were highly statistically significant at all time points (p<0.00001).
4.2.10 BAP responds to antiresorptive therapy as early as 8 weeks

A study of two bisphosphonates was undertaken to evaluate short-term changes in marker of bone turnover. 74 postmenopausal women with osteopenia were randomized to receive calcium carbonate (500 mg/day), cyclical etidronate (cyc ETID), or alendronate 5 or 10 mg/day (ALN 5,ALN 10). BAP was measured at baseline and following 2, 4, 8, and 12 weeks of therapy. There is a lag in the response to anti-resorptive therapies of bone formation marker like BAP compared with bone resorption marker. This is a reflection of the timing of the bone remodeling cycle. Decreases in osteoblast activity cannot occur until the primary osteoclastic effect of anti-resorptive therapies has occurred. Despite this time lag, BAP proved to be sensitive to the effects of the higher dose of alendronate as early as 8 weeks. By 12 weeks, BAP had decreased significantly (p<0.05) in response to both alendronate 10 mg/day and cyclical etidronate [19].

4.2.11 BAP is more sensitive than total alkaline phosphatase to bisphosphonates in Paget’s disease

BAP offers a more sensitive measure of changes in bone turnover than total alkaline phosphatase activity (TAP), even in Paget’s disease where increases in TAP are almost entirely attributable to the bone isoenzyme. Bisphosphonate rapidly suppresses the high bone turnover of Paget’s disease as reflected in significant decreases in both BAP and TAP. Decreases in BAP are significantly greater than decreases in TAP. These data are from two studies [15, 23]. One included 12 previously untreated pagetic patients measured at baseline and 3 months after intravenous administration of clodronate or alendronate. The other included 13 previously untreated patients with mild disease treated with daily etidronate 400 mg.

4.2.12 BAP and strontium ranelate therapy

Strontium ranelate is a promising new drug that inhibits bone resorption and stimulates bone formation [40]. In a study by Meunier [13] 1649 osteoporotic women with low bone density and at least one fracture were treated daily with 2 g of oral strontium ranelate. An increase of bone mineral density (14.4 percent at the lumbar spine) and a reduction of fractures (41% reduction) were observed as compared to the placebo group after 3 years of treatment. The dissociation of bone resorption and bone formation was demonstrated with biochemical marker CTX-I and BAP. BAP was higher (treatment-related increase of 8.1 %) and CTX-I was lower (treatment-related decrease of 12.2 %) in the strontium ranelate group than in the placebo group. However, these relative small changes of bone marker in response to strontium ranelate therapy suggest that they are less optimal for monitoring the efficacy of this therapy.
4.2.13 BAP and parathyroid hormone treatment

In contrast to anti-resorptive agents, which reduce bone remodeling, PTH initially stimulates bone formation and later increases bone remodeling. **Bone formation marker increase within one month, whereas bone resorption marker increase within 6 months.**

In a recent study [10] osteoporotic patients on long-term alendronate therapy, were also given recombinant human PTH (1-34). Daily PTH treatment induced an increment in spinal BMD of 6.1%. An increase of >30% in bone turnover marker at 3 months had a predictive value of more than 73% for an increase in spinal BMD of at least 3%. Over 15 months of therapy, BAP values had more than doubled (+116% see Figure). Bone resorption marker NTX-I rose more slowly and to a lesser extent.

4.2.14 Measurement of BAP in cancer

**BAP can differentiate between tumor patients with and without bone metastases.**

A total of 39 tumor patients were examined for BAP levels in a study by Withold et al. [27]. In patients with bone metastases (15), BAP levels were strongly increased as compared with a sex-matched reference group (Z-score: 7.3), whereas in tumor patients without bone metastases BAP levels were not significantly different from the reference group (Z-score: -0.3). Patients with multiple myeloma showed markedly decreased values of BAP (Z-score: -1.5). (Z scores were calculated according to formula \((x_i - M)/SD\), where \(x_i\) is the value of an individual patient, \(M\) is the mean of the reference group and \(SD\) is standard deviation of the reference group.)

**Prostate cancer: BAP is useful for monitoring patients with bone metastases.**

In a retrospective study [34] on the interrelationship of serum bone alkaline phosphatase (BAP) and prostate-specific antigen (PSA) in 156 patients with M0 and M1 prostate cancer, BAP demonstrated to be a more sensitive and more specific method of determining osteoblast activity than total alkaline phosphatase (TAP). A raised BAP was observed in 86.4% of M1 disease at diagnosis before treatment. The change of BAP was concordant with PSA in 69% of 49 cases of M1 disease.
Breast, prostate and lung cancer: BAP is useful for detecting bone metastases.

A recent study by Leeming et al. [39] investigated the relative use of biochemical bone marker for the detection of bone metastases in cancer forms frequently spreading to the skeleton. Participants were 161 patients with breast, prostate, or lung cancer. Expressing sensitivity as the percentage increase in marker value relative to patients without bone metastases (Soloway score 0), CTX showed the largest relative increases at each stage of the metastatic disease.

Bone formation marker BAP was most indicative for the presence of bone metastases in prostate cancer patients.

4.2.15 BAP and growth hormone therapy

Elmlinger et al. [25] demonstrated that patients with disorders (GH deficiency, idiopathic short stature and Ullrich-Turner syndrome) showed significantly decreased BAP and PICP=CICP serum levels before growth hormone therapy. Bone parameters increased up to normal levels after 12 months of GH therapy. It is concluded that BAP and PCIP are valid parameters to monitor the efficacy of GH-therapy. Walmsley et al. [26] observed elevated BAP levels following 3 months of GH therapy in adult patients suffering from hypopituitarism. No change in BMD was seen at this early stage of treatment.

4.2.16 BAP in uremic patients

Chronic renal failure is often associated with bone disorders, including secondary hyperparathyroidism, aluminum-related low-turnover bone disease, osteomalacia, adynamic osteopathy, osteoporosis, and skeletal beta2-microglobulin amyloid deposits.

Urena et al. [29] made an in-depth review of the diagnostic value of serum marker of bone metabolism in patients with chronic renal failure. The investigation included BAP, procollagen type I carboxy-terminal propeptide (PICP), procollagen type I cross-linked carboxy-terminal telopeptide (ICTP), pyridinoline (PYD), osteocalcin, and tartrate-resistant acid phosphatase (TRAP 5b).

Most of the observations made by several groups converged to the conclusion that serum BAP is one of the most sensitive and specific marker to evaluate the degree of bone remodeling in uremic patients.

4.2.17 BAP is more sensitive than total alkaline phosphatase in primary hyperparathyroidism

TAP is not sensitive enough in primary hyperparathyroidism, especially in asymptomatic situations. In contrast, BAP levels are significantly elevated in both symptomatic and asymptomatic primary hyperparathyroidism [48].
4.2.18 BAP is elevated in secondary hyperparathyroidism

BAP is not cleared by the kidney. However, in secondary hyperparathyroidism, as caused by chronic renal failure, levels of BAP are elevated up to 3-fold. Moreover, BAP and PTH (parathyroid hormone) levels are correlated [31]. BAP is therefore a useful marker for bone turnover in secondary hyperparathyroidism caused by chronic renal failure. This is especially relevant for dialysis patients.

4.2.19 BAP is elevated in Morbus Paget's disease

In patients with Morbus Paget's disease, levels of BAP can be elevated up to 16-fold of the values prior to disease [31]. In a study by Deftos et al. it was demonstrated that BAP was increased in the serum of patients with Paget's disease. Comparisons with TAP and Osteocalcin measurements revealed that BAP correlated better with TAP ($r = 0.92$) than with Osteocalcin ($r = 0.51$); the correlation between Osteocalcin and TAP was only ($r = 0.26$). In patients with liver disease, BAP was indistinguishable from normal whereas the TAP was elevated [31, 32].

The use of BAP is advised by the German Endocrinology Society for the diagnosis of Morbus Paget's disease [33].

4.2.20 Determination of BAP in patients suffering from severe liver disease

Due to cross-reaction of the BAP antibody with the liver isoenzyme (6–8 %), measured BAP levels could be erroneously elevated in case of severe liver disease (e.g. liver carcinoma or liver cirrhosis where liver alkaline phosphatase is highly elevated, see also Fig. 2d) [35]. This can also occur in case of bile duct cancer. To assess bone turnover in these clinical situations, where total alkaline phosphatase levels are above 270 U/L, it was advised to determine osteocalcin levels [31].
4.3 BAP reference data

The Quidel® BAP immunoassay provides a quantitative measure of bone-specific alkaline phosphatase (BAP) activity in serum as an indicator of osteoblastic activity.

Measurement of BAP is intended for use as an aid in:
- management of postmenopausal osteoporosis and Paget’s disease
- monitoring of postmenopausal women on hormonal or bisphosphonate therapy
- prediction of skeletal response to hormonal therapy in postmenopausal women

Reference data for the Quidel® BAP EIA were generated using data from individuals carefully selected. Age related BAP values for pre- and postmenopausal women [46] are shown in Fig. 1. Distribution of the BAP values in both groups [28] is shown in Fig. 2. In general, women in the postmenopausal group showed higher levels of BAP relative to their normal, premenopausal counterparts.

Fig. 1. BAP values in pre- and postmenopausal women

![Fig. 1. BAP values in pre- and postmenopausal women](image)

Fig. 2. Distribution of BAP levels in pre- and postmenopausal women

![Fig. 2. Distribution of BAP levels in pre- and postmenopausal women](image)

<table>
<thead>
<tr>
<th>N</th>
<th>Age Group</th>
<th>Type</th>
<th>Reference Range (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>Females ( Age: 25-44 yrs.)</td>
<td>Premenopausal</td>
<td>11.6–29.6</td>
</tr>
<tr>
<td>107</td>
<td>Females ( Age: ≥ 45 yrs.)</td>
<td>Postmenopausal</td>
<td>14.2–42.7</td>
</tr>
<tr>
<td>178</td>
<td>Males (≥ 25 yrs.)</td>
<td>N/A</td>
<td>15.0–41.3</td>
</tr>
</tbody>
</table>

Table 1. Reference Ranges for BAP (non-parametric, 90% confidence intervals).
4.3.1 BAP reference data in premenopausal women

Reference data for the Quidel® BAP EIA were generated using data from individuals selected to have no bone, endocrine or other conditions known to affect bone metabolism. As with other bone marker, premenopausal females are used for normal comparative purposes. 191 premenopausal females age 25 to 44 years were tested; the results are presented in Table 1 and Fig. 3. Using these reference data, the normal premenopausal cut off value for BAP would be 30 U/L or 15 μg/L. In subjects at the low and high end of the age range, elevation of mean BAP is seen as expected.

![Fig. 3. BAP values in Premenopausal Women](image1)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BAP (U/L)</td>
<td>21.7</td>
<td>19.4</td>
<td>18.9</td>
<td>22.0</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>34</td>
<td>93</td>
<td>60</td>
<td>4</td>
</tr>
</tbody>
</table>

4.3.2 BAP reference data in postmenopausal women

BAP was measured in 107 postmenopausal females (age 44-88); the results are presented in Table 1 and Fig. 4. Mean BAP values were substantially higher, indicating higher levels of bone remodeling occurring in this age group versus normal, premenopausal controls (see Fig. 1 and 2). However, a single BAP measurement, either within or above the premenopausal reference range, is not necessarily an indication of presence or absence of osteoporosis in postmenopausal women. Osteoporosis should always be diagnosed in conjunction with Bone Mineral Density (BMD) measurements.

![Fig. 4. BAP values in Postmenopausal Women](image2)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70-79</th>
<th>80-89</th>
<th>90-99</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BAP (U/L)</td>
<td>18.6</td>
<td>24.8</td>
<td>25.7</td>
<td>26.4</td>
<td>25.8</td>
<td>33.0</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>5</td>
<td>27</td>
<td>25</td>
<td>38</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>
4.3.3 BAP in postmenopausal osteoporotic women on hormonal replacement or bisphosphonate therapy

Postmenopausal women diagnosed with osteoporosis can be treated with hormonal replacement (HRT) or bisphosphonates. To identify therapy responders and non-responders the International Osteoporosis Foundation [47] has formulated the following recommendation:

For a 90% specificity to predict a positive BMD response (+3%), the BAP value of an individual patient should decrease 20 to 40% from the baseline value (see Fig. 5) [17]. In order to control the therapy efficacy BAP should be measured on a regular basis.

![BAP response in postmenopausal women on HRT therapy](image)

4.3.4 BAP reference data in males

BAP was measured in 126 males (age 25–91); the results are presented in Table 1 and Fig. 6. BAP values in males show less change with age than those of females and are generally slightly higher than premenopausal female controls.

![BAP values in males](image)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean BAP (U/L)</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>25.7</td>
<td>17</td>
</tr>
<tr>
<td>30-39</td>
<td>25.0</td>
<td>39</td>
</tr>
<tr>
<td>40-49</td>
<td>23.4</td>
<td>24</td>
</tr>
<tr>
<td>50-59</td>
<td>29.5</td>
<td>4</td>
</tr>
<tr>
<td>60-69</td>
<td>24.6</td>
<td>20</td>
</tr>
<tr>
<td>70-79</td>
<td>24.7</td>
<td>22</td>
</tr>
<tr>
<td>&gt; 80</td>
<td>34.3</td>
<td>4</td>
</tr>
</tbody>
</table>
### 4.3.5 BAP reference data in children

A total of 424 healthy children, adolescents and young adults (221 male) aged 0.1 to 21 years were enrolled in a pediatric reference range study [49] for determination of c-terminal fragment of fibroblast growth factor-23 (cFGF-23), sclerostin, bone alkaline phosphatase (BAP), and tartrate-resistant acid phosphatase 5b (TRAP5b). BAP was measured in plasma/serum samples from 352 individuals. Values are expressed in U/L, ranges as 3rd to 97th percentile.

As with other bone marker, values for BAP in children are elevated over normal premenopausal controls.

<table>
<thead>
<tr>
<th>Children, female (U/L)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td><strong>N</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Percentile Range (3rd - 97th %)</strong></td>
</tr>
<tr>
<td>&lt;1</td>
<td>3</td>
<td>135</td>
<td>79-178</td>
</tr>
<tr>
<td>2-4</td>
<td>20</td>
<td>129</td>
<td>77-180</td>
</tr>
<tr>
<td>5-11</td>
<td>48</td>
<td>118</td>
<td>70-200</td>
</tr>
<tr>
<td>12-15</td>
<td>58</td>
<td>67</td>
<td>36-146</td>
</tr>
<tr>
<td>16-19</td>
<td>38</td>
<td>26</td>
<td>11-56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Children, male (U/L)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td><strong>N</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Percentile Range (3rd - 97th %)</strong></td>
</tr>
<tr>
<td>&lt;1</td>
<td>9</td>
<td>126</td>
<td>77-168</td>
</tr>
<tr>
<td>2-4</td>
<td>18</td>
<td>115</td>
<td>68-157</td>
</tr>
<tr>
<td>5-11</td>
<td>74</td>
<td>114</td>
<td>59-165</td>
</tr>
<tr>
<td>12-15</td>
<td>34</td>
<td>114</td>
<td>46-192</td>
</tr>
<tr>
<td>16-19</td>
<td>44</td>
<td>57</td>
<td>22-112</td>
</tr>
</tbody>
</table>

### 4.4 BAP disease values

<table>
<thead>
<tr>
<th>Disease</th>
<th>BAP levels compared to normal reference group</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoporosis</td>
<td>▲</td>
<td>Median 18 U/L (25th-75th percentiles: 16-22 U/L) [24]</td>
</tr>
<tr>
<td>Primary hyperparathyroidism</td>
<td>▲</td>
<td>NA</td>
</tr>
<tr>
<td>Paget's</td>
<td>▲</td>
<td>Median 91 U/L (25th-75th percentiles: 45-217 U/L) [24]</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>▲</td>
<td>Median 65 U/L [30]</td>
</tr>
<tr>
<td>Bone metastases</td>
<td>▲</td>
<td>Z-score value is 7.3 ± 1.9 (SD) [29]</td>
</tr>
<tr>
<td>Growth hormone deficiency</td>
<td>▼</td>
<td>Majority below 50th percentile of normal age group, majority of Ullrich-Turner syndrome girls is below 5th percentile [27]</td>
</tr>
<tr>
<td>Rheumatoid arthritis in postmenopausal women</td>
<td>▼</td>
<td>Decreased compared to normal post-menopausal women</td>
</tr>
<tr>
<td>Renal transplantation</td>
<td>▲</td>
<td>Increased values within 3 months after transplantation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Therapy</th>
<th>BAP levels compared to reference group before therapy</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone replacement</td>
<td>▲</td>
<td></td>
</tr>
<tr>
<td>Bisphosphonates</td>
<td>▼</td>
<td></td>
</tr>
<tr>
<td>PTH analogues</td>
<td>▲</td>
<td>In postmenopausal women with glucocorticoid-induced osteoporosis [23]</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>▲</td>
<td>Compared to low levels before onset of GH therapy [27]</td>
</tr>
<tr>
<td>Strontium ranelate</td>
<td>▲</td>
<td></td>
</tr>
</tbody>
</table>
5 Clinical validation studies of the Quidel® BAP Assays

Clinical studies. Use of Quidel® BAP for Monitoring the Efficacy of Antiresorptive Therapy in Osteoporosis.

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the Quidel® BAP assay to monitor changes in serum BAP concentrations associated with amino-bisphosphonate (alendronate) antiresorptive therapy. Subjects, drawn from a larger study of the efficacy of alendronate for treating osteoporosis [7], were postmenopausal women, aged 45 to 84 years (mean 64 ± 7 years), diagnosed with osteoporosis (based on clinical presentation or baseline lumbar spine bone mineral density [LSBMD] more than 2.5 standard deviations below the mean for mature premenopausal women). At baseline, eligible subjects were randomized to receive either 10 mg alendronate and 500 mg calcium per day (ALN) or placebo and 500 mg calcium per day (CTL). Serum specimens were obtained at baseline, 3, 6 and 12 months from all subjects. Mean (± 1SD) baseline BAP concentration (14.6 ± 5.4 vs. 14.6 ± 4.6, p = 0.900) and LSBMD (0.74 ± 0.10 vs. 0.75 ± 0.09, p = 0.751) were similar values for ALN and CTL. Distributions of baseline BAP values in ALN and CTL are depicted in the following figure by proportion of the study population.

Distribution of BAP Levels At Baseline

BAP was significantly lower for ALN than CTL at 3 (9.6 ± 3.5 vs. 13.4 ± 4.0, p<0.00001), 6 (8.0 ± 3.0 vs. 13.2 ± 3.8, p<0.00001), and 12 months (7.8 ± 2.6 vs. 13.3 ± 3.9, p<0.00001). Distributions of BAP values following 12 months in the ALN and CTL groups are depicted in the figure.

Distribution of BAP Levels Following 12 Months Therapy with Alendronate (ALN) or Calcium (CTL)

The mean (± 1SD) BAP concentration in CTL subjects decreased modestly from baseline to -5.4 % (± 19.1%) at 12 months (p=0.00004) which may reflect the limited bone-sparing effect of calcium [13]. Mean BAP concentrations in ALN subjects decreased 30.5 ± 24.6 % at 3 months, 42.8 ± 17.3 % at 6 months, and 42.2 ± 19.2 % at 12 months. Subjects in ALN were more likely than CTL subjects to demonstrate BAP losses exceeding minimum percent change [14] with 68.5 %, 83.9 %, and 86.1 % of ALN and 9.5 %, 15.9 % and 9.0 % of CTL individuals decreasing by ≥ 25 % at the 3, 6, and 12 month timepoints. Distributions of the percent change from baseline in BAP values following 12 months in the ALN or CTL groups are depicted in the figure.
Distribution of Percent Change in BAP Levels Following 12 Months Therapy with Alendronate (ALN) or Calcium (CTL)

At 12 months, subjects in ALN had gained LSBMD compared to CTL \((p < 0.00001)\) as shown in the following table.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Baseline (g/cm²)</th>
<th>12 months (g/cm²)</th>
<th>∆(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>159</td>
<td>0.75 ± 0.09</td>
<td>0.74 ± 0.09</td>
<td>-0.6 ± 3.4</td>
</tr>
<tr>
<td>ALN</td>
<td>121</td>
<td>0.74 ± 0.10</td>
<td>0.79 ± 0.10</td>
<td>5.5 ± 4.1</td>
</tr>
</tbody>
</table>

These results indicate that the Quidel® BAP assay is safe and effective for monitoring the antiresorptive effect of aminobis-phosphonate (alendronate) therapy among subjects diagnosed with osteoporosis.

Use of Quidel® BAP for Monitoring Hormonal Antiresorptive Therapy and Predicting Skeletal Response (Bone Mineral Density) in Postmenopausal Women

Monitoring Therapy:
A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the Quidel® BAP assay to monitor the changes in serum BAP concentrations associated with estrogen/progestin antiresorptive therapy. Increased bone turnover and significant loss of bone are often associated with postmenopausal estrogen deficiency. Estrogen replacement has been shown to effectively decrease bone turnover and protect existing bone mass [3, 6]. Subjects were postmenopausal women, aged 45 to 64 years (mean 56 ± 4 years), who had undergone natural or surgical menopause within the last 10 years. At baseline, eligible subjects were randomized to either an active treatment group (HRT): Premarin® (0.625 mg daily) with placebo progestin, Premarin® (0.625 mg daily) and an active progestin (Provera® 2.5 mg/day continuous, Provera® 10 mg/day cyclical, or micronized progesterone 200 mg/day cyclical); or to the control group (CTL): placebo estrogen and placebo progestin. Serum specimens were obtained at baseline and 12 months from all subjects. Mean (± 1SD) baseline BAP concentration (20.7 ± 7.6 vs. 20.3 ± 6.8 U/L, \(p = 0.704\)) and LSBMD (0.97 ± 0.17 vs. 0.97 ± 0.15 g/cm², \(p = 0.970\)) were similar for CTL and HRT. Distributions of baseline BAP values in HRT and CTL are depicted in the following figure by proportion of the study population.

Distribution of BAP Levels At Baseline
BAP was significantly lower for HRT than CTL at 12 months (13.3 ± 5.0 vs. 21.9 ± 7.9 U/L, \(p < 0.00001\)). Distributions of BAP values following 12 months in the HRT and CTL groups are depicted in the following figure.
Distribution of BAP Levels Following 12 Months Therapy with Estrogen/Progestin (HRT) or Placebo (CTL)

The mean (± 1SD) BAP concentration in CTL subjects increased slightly from baseline to +9.8 % (± 33.2 %) at 12 months (p = 0.08) whereas BAP concentrations in HRT subjects decreased from baseline to -32.4 (± 21.5 %) at 12 months (p < 0.00001). Subjects in HRT were more likely than CTL subjects to demonstrate BAP losses exceeding minimum percent change. 12 with 73.3 % of HRT and 3.4 % of CTL individuals decreasing by ≥ 25 % at the 12 month timepoint. Distributions of the percent change from baseline in BAP values following 12 months in the HRT and CTL groups are depicted in the following figure.

Distribution of Percent Change in BAP Levels Following 12 Months Therapy with Estrogen/Progestin (HRT) or Placebo (CTL)

At 12 months, subjects in HRT had gained LSBMD compared to CTL (p<0.00001) as shown in the following table.

<table>
<thead>
<tr>
<th>Changes in LSBMD (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>CTL</td>
</tr>
<tr>
<td>HRT</td>
</tr>
</tbody>
</table>

These results indicate that the Quidel® BAP assay is safe and effective for monitoring the antiresorptive effect of hormone replacement therapy in postmenopausal women.

Predicting Skeletal Response:

The following figure depicts the % decrease in BAP values from baseline to 12 months by quartile for the HRT-treated group. Subjects in the highest quartile (Q1: greatest % decrease) showed the greatest gain in LSBMD in response to HRT.

HRT Group - Values of % Change in BAP to 12 months stratified by Quartile and Corresponding % Change in LSBMD at 12 months

The following figure provides the linear regression analysis (y = -0.060x + 0.011, r = -0.51, p < 0.001) of the percent change from baseline to 12 months BAP and percent change from baseline to 12 months BMD for all subjects in the study (placebo and treated).
Contingency table analysis showed that a ≥ 25 % decrease in BAP at 12 months was significantly associated (p < 0.0001) with a positive skeletal response to HRT (gain in BMD) at 12 months. The binomial (second order approximation) 85 % confidence intervals for the sensitivity and specificity of using a 25 % decrease in BAP for predicting a response to HRT are:

Sensitivity = 77 % (95 % CI 75 %, 82 %)
Specificity = 61 % (95 % CI 41 %, 78 %)

These results indicate that the % change in BAP concentration can be used to predict the degree of skeletal response (BMD) to HRT treatment.

References (section 5)


6 Special applications of the Quidel® BAP Assays

6.1 Use of BAP with cell culture supernatant

Recommendation: culture medium with the following components:
- DMEM or equivalent commercial medium
- Foetal Bovine Serum or Calf Serum
- L-glutamine or ascorbate
- Antibiotic

The cells can be grown in serum containing media, but BAP must be harvested from tissue culture supernatant that is serum-free, as the antibody will also detect these analytes in serum. Ion chelaters, such as EDTA, should not be used in the media, because chelators are inhibiting the BAP enzyme. Sheep and human cells can be used for this purpose, but not mouse, as the antibody does not cross react with this species.

Bone alkaline phosphatase is membrane-bound and it needs to be measured in culture from cell lysate according to following procedure [30]:

After incubation, cell layers are washed twice with saline and harvested by scraping with a stirring stick into TMN buffer (20 mM Tris-HCL, pH 7.4; 2 mM MgCl2; 150 mM NaCl). Cell number can be determined by a Coulter counter (model F) before the cells are solubilized by the addition of Triton X-100 to a final concentration of 1 %. The samples are centrifuged at 70,000.-g for 60 minutes and aliquots of the supernatant can be assayed for BAP.

6.2 Use of BAP monoclonal antibody in western blotting, FACS sorting and Immunocytochemical staining of osteoblasts

The BAP monoclonal antibody present in the Quidel® BAP assay can be used in several research methods like Western Blotting, Fluorescence Activated Cell Sorter (FACS) and immunocytochemical and histochemical staining of bone alkaline phosphatase in osteoblasts.

Enzymehistochemical results for bone alkaline phosphatase in subconfluent primary human osteoblasts (a) and HOS 58 osteosarcoma cells (c).

Photographs are kindly provided by:
Prof. Heide Siggelkow University Göttingen, Germany

FACS sorting and analysis.
Subpopulations of human osteoblast cell cultures were isolated by means of FACS sorting. Purity of the fractions was checked using FACS analysis. Staining was performed using the Quidel® Quidel BAP monoclonal antibody.
6.3 Measurement of BAP in animal species

The BAP assay shows cross reaction with following animal species:
- Bovine
- Cat
- Dog
- Horse
- Goat
- Pig
- Macaque monkey
- Rabbit
- Sheep

7 Technical summary of the Quidel® BAP Assay

(BAP) Bone specific alkaline phosphatase  
For In Vitro Diagnostic Use

Enzyme activity of bound BAP is measured.

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
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<tbody>
<tr>
<td>Catalog Nr</td>
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<tr>
<td>Range</td>
<td>2–140 U/L resp. 1–70 μ/l</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.7 U/L</td>
</tr>
<tr>
<td>Incubation time</td>
<td>3.5 hours</td>
</tr>
<tr>
<td>Specimen volume</td>
<td>20 μl</td>
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<tr>
<td>Pipetting steps</td>
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</tr>
<tr>
<td>Specimen type</td>
<td>Heparin-Plasma, Serum, cell culture</td>
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</tbody>
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Sample pre-treatment:
Plasma samples should not be treated with EDTA or citrate. Storage of samples for a maximum of 5 days at 2–8 ºC. For longer storage, samples should be kept frozen at -20 ºC or below.

Cross-reaction:
Minimal cross-reaction (6–8 %) with Liver alkaline phosphatase.
8 Measurement of BAP in protein mass (μg/L) and enzyme activity (U/L)

Bone Alkaline Phosphatase (BAP) is a marker for osteoblastic activity in vitro. BAP is typically measured by one of the two methods, protein mass or enzyme activity.

Enzyme Activity

BAP is bound to a monoclonal antibody specific for the bone isoform. Other forms of Alkaline Phosphatase (e.g. liver, etc) are washed away. Alkaline Phosphatase activity is measured via a chromogenic (color change), chemical reaction. In the Quidel® BAP Assay, results are determined directly from this color change and expressed as units per liter (u/L).

Protein Mass

In the IRMA method, the enzyme molecule BAP is directly measured by using two monoclonal antibodies (Sandwich Assay) detecting two different epitopes; results are expressed in mass units (μg/L).

Correlation Enzyme activity and Protein Mass expressed in U/L and μg/L

In order to establish correlation between the mass and enzymatic methods, serum samples have been measured with both assays in different independent reference centers. In all cases the samples showed very good correlation between the two assays across the dynamic range of both methods.
Correlation Protein Mass and Enzyme activity expressed in μg/L

BAP measured with the protein mass and the enzyme activity method, both expressed in μg/L showed an excellent correlation.

<table>
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<tr>
<th>Children Reference values Quidel BAP in U/L and μg/L [49]</th>
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</tr>
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<td>Age</td>
<td>N</td>
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<td>&lt;1</td>
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<tr>
<td>12-15</td>
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<td>16-19</td>
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<table>
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<tr>
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<table>
<thead>
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<tr>
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<td>18-24</td>
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<tr>
<td>25-44 premenopausal</td>
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<tr>
<td>≥ 45 postmenopausal</td>
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<tr>
<td>Age</td>
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<tr>
<td>19-24</td>
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<tr>
<td>≥ 24</td>
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<table>
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<tr>
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<td>μg/L ± SD</td>
<td>U/L ± SD</td>
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<tr>
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<td>30</td>
<td>95 ± 22</td>
<td>47.5 ± 11.0</td>
</tr>
<tr>
<td>f</td>
<td>26</td>
<td>84 ± 23</td>
<td>42.0 ± 11.5</td>
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8.1 Certificate of Analysis / Analysenzertifikat / Certificat d’analyse

Quidel® BAP Kit
Ref. 8012
LOT 904151 Exp. 2008-10

Refer to Product Insert for further information.
Weitere Informationen hierzu finden Sie in der Packungsbeilage des Produkts.
Se référer à la Notice produit pour plus d’information.

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<table>
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<tr>
<th>BAP Control</th>
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<tbody>
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