marketplace vagaries. It is reasonable that the dramatic changes in the healthcare industry have prompted the clinical laboratory to choose automated cost-saving methods. Although the advantages of analytical precision and accuracy can certainly be debated [7–9], it is evident that clinicians must be aware of these seldom-explored issues as they affect patient care. Establishment of method-dependent reference intervals will minimize this type of discrepancy. Future resolutions may require improved assay standardization and use of an international reference material.

References


Bone alkaline phosphatase (b-ALP; EC 3.1.3.1) is a tetrameric glycoprotein found on the cell surface of osteoblasts. Previous studies have shown that both b-ALP [1] and total ALP [2] can be considered markers of bone formation. Because postmenopausal osteoporosis in women and senile osteoporosis in both sexes are global epidemiological problems, biochemical markers of bone turnover have become important clinical tests. Such markers are used to identify subjects with rapid bone turnover (and thus bone loss) [3] and to monitor the therapeutic efficacy of various drugs [4]. These two purposes mandate separate reference ranges for different sexes and age groups. To evaluate the age-related changes of these two markers in Chinese men and women, we measured both total ALP and b-ALP of healthy Chinese men (n = 156, ages 20–90 years) and women (n = 385, ages 40–70 years; 118 premenopausal and 267 postmenopausal), all of whom underwent a careful screening and showed no liver or bone disease.

Total serum ALP activity was analyzed with a Hitachi-7450 automated analyzer and diphosphocresol phosphate as the substrate (Hitachi, Tokyo, Japan). The procedure has a long-term (over 1 year) imprecision

![Fig. 1. (A) Mean plasma folate concentration obtained by automated (open bars) and manual (hatched bars) methods for nine CAP survey samples (error bars, standard deviation); (B) correlation graph of automated vs manual mean plasma folate concentration (best-fit regression line shown); (C) number of laboratories reporting automated (open bars) vs manual (hatched bars) plasma folate methods during 1995 CAP survey challenge (percentage of total laboratories participating shown).

Table 1. Comparison of mean plasma folate concentrations obtained by automated vs manual methods.

<table>
<thead>
<tr>
<th>Survey sample, K</th>
<th>x_{auto} - x_{man} µg/L</th>
<th>Difference, %</th>
<th>Discrepancy rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>4.39</td>
<td>144</td>
<td>3/10</td>
</tr>
<tr>
<td>02</td>
<td>1.50</td>
<td>264</td>
<td>1/10</td>
</tr>
<tr>
<td>03</td>
<td>3.60</td>
<td>169</td>
<td>2/10</td>
</tr>
<tr>
<td>06</td>
<td>1.61</td>
<td>236</td>
<td>3/9</td>
</tr>
<tr>
<td>07</td>
<td>7.06</td>
<td>178</td>
<td>1/10</td>
</tr>
<tr>
<td>08</td>
<td>4.22</td>
<td>186</td>
<td>1/10</td>
</tr>
<tr>
<td>11</td>
<td>2.84</td>
<td>190</td>
<td>1/9</td>
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<tr>
<td>12</td>
<td>2.81</td>
<td>189</td>
<td>1/9</td>
</tr>
<tr>
<td>13</td>
<td>4.36</td>
<td>187</td>
<td>1/9</td>
</tr>
</tbody>
</table>

* 3rd most discrepant (comparison of means) of 10 methods reporting.
b-ALP was measured with a commercial kit (Alkphase-B™; Metra Biosystems, Los Angeles, CA) that utilized a monoclonal anti-b-ALP antibody coated on a microtiter plate to capture b-ALP in the samples; the enzyme activity of the captured b-ALP was then detected by using p-nitrophenyl phosphate substrate \[5\]. The intra- and interassay CVs in our laboratory were 8% and 11%, respectively, for b-ALP at 25 U/L. We then adjusted the reading of total ALP (Hitachi ALP) to the reading obtained on the microtiter plate and with p-nitrophenyl phosphate substrate (plate ALP), according to a published method \[6\]; in our laboratory, Hitachi ALP = 2.93 plate ALP + 12.57 \(r = 0.97, P < 0.0001\). We then derived the nonbone fraction of ALP by subtracting b-ALP from plate ALP activity.

The results (Fig. 1) showed increased b-ALP activity in women and a borderline decrease of b-ALP in men \(P = 0.015\) with aging. In contrast, the nonbone form (assumed to be largely the liver form) of ALP showed the same trends of increase in both men and women, but the slope of the regression line was slightly steeper for the men’s results. The total ALP activity showed a borderline increase \(P = 0.088\) in men and a significant \(P = 0.0001\) increase in women with increasing age. The proposed age-specific reference intervals (central 95% percentiles) for men and women, premenopausal and postmenopausal, are given in Table 1.

Our findings suggested that bone formation rates—as reflected by b-ALP, a specific bone formation marker—decreased rather than increased in elderly men. This is compatible with the findings of decreases in markers of bone formation in the histomorphometry of bone in older men \[7\] and with previous reports of decreased bone formation markers including b-ALP \[8\] and osteocalcin \[7, 8\] in elderly men. Our results were also compatible with a previous study of elderly or postmenopausal women, which showed increased turnover rate of bone in both histology markers \[7\] and biochemical bone markers \[2, 3\].

The increase of b-ALP after menopause in women has been explained by removal of the inhibitory effects of estrogen on turnover rate \[9\]. At present, the relation

\[
\begin{align*}
\text{MEN} & \quad y = 0.1x + 33.5 \\
\text{WOMEN} & \quad y = 0.5x + 5.6 \\
\end{align*}
\]

\[
\begin{align*}
y & = 0.08 + 23.0 \\
y & = 0.4x + 3.0 \\
\end{align*}
\]

\[
\begin{align*}
y & = 0.2x + 10.8 \\
y & = 0.16x + 4.9 \\
\end{align*}
\]

**Fig. 1.** Linear regression of total ALP (plate method; see text), b-ALP, and nonbone ALP (in U/L) vs age in healthy Chinese men and women. (●) Premenopausal; (○) postmenopausal women. The two r values at the left upper corners of the panels for women are for pre- and postmenopausal women separately.
between the decrease of b-ALP and testosterone concentrations in elderly men is controversial [10]. Other bone markers, including osteocalcin and carboxyl-terminal propeptide of type I procollagen, have been shown to correlate poorly with testosterone concentrations in elderly men [8, 10], and increased nonbone ALP may in part represent subclinical liver diseases or a decreased clearance of ALP in elderly men and women.

In conclusion, because total ALP and b-ALP showed in men opposite trends of age-related changes, our results suggest that total ALP is not a good marker of bone turnover, especially for men.

References

Table 1. Age-specific reference intervals for total ALP (plate method), b-ALP, and nonbone ALP in healthy Chinese men and women.

<table>
<thead>
<tr>
<th>Ages</th>
<th>Reference intervals (mean ± 2 SD), U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total ALP</td>
</tr>
<tr>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>20–40 years</td>
<td>24–66</td>
</tr>
<tr>
<td>41–60 years</td>
<td>22–62</td>
</tr>
<tr>
<td>61–90 years</td>
<td>26–76</td>
</tr>
<tr>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>18.5–39.5</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>21.5–65</td>
</tr>
</tbody>
</table>