by Passing–Bablok analysis showed that results obtained with the two chromogens were correlated. The Bland–Altman difference plot (Fig. 1) showed that eight of the paired values were outside of the 95% confidence interval (−2.6 and 4.2). These values fell nearly within the limits of agreement, and medical acceptance remained possible.

The determination of low HIC with ferene gave better sensitivity (0.333 vs 0.215) and a lower detection limit (0.15 vs 0.30 μmol/L) than with bathophenanthroline. Moreover, this chromogen is less expensive than bathophenanthroline.

In conclusion, ferene, which is less expensive than bathophenanthroline sulfonate, slightly improves the sensitivity of the colorimetric measurement of low HIC. Ferene can beneficially replace bathophenanthroline sulfonate in HIC determinations as recommended by the ICSH for serum iron (8).

Osteoprotegerin in Serum as a Novel Marker of Bone Metastatic Spread in Prostate Cancer, Klaus Jung,1* Michael Lein,1 Katharina von Hösslin,1 Brigitte Bruck,2 Dietmar Schnorr,1 Stefan A. Loening,1 and Pranav Sinha2 (Departments of 1 Urology and 2 Laboratory Medicine, University Hospital Charité, Humboldt University Berlin, D-10098 Berlin, Germany; * address correspondence to this author at: Department of Urology, Research Division, University Hospital Charité, Humboldt University, Schumannstrasse 20/21, D-10098 Berlin, Germany; fax 4930-450-515904, e-mail klaus.jung@charite.de)

Prostate cancer (PCa) is the most frequent carcinoma in men and is often complicated by skeletal metastasis (1). Because bone scintigraphy, the standard method of monitoring metastatic bone involvement, is expensive, lacks specificity, and is not particularly suitable for the follow-up of patients, various metabolic bone markers have been studied as indicators for bone metastasis in PCa patients (2, 3). Markers that reflect osteoblast proliferation, e.g., skeletal alkaline phosphatase (sALP), are reportedly useful, which is consistent with the osteoblastic reactions seen in the skeletal metastases (4).

The balance between osteoblastic and osteoclastic activity in bone is essentially influenced by osteoclastogenesis. The latter is regulated by three proteins: receptor activator of nuclear factor-kB (RANK), which is expressed on osteoclast precursor cells; its ligand (RANKL), which is expressed on the surface of preosteoblastic and stromal cells; and osteoprotegerin (5, 6). The interaction of RANKL with RANK stimulates the differentiation of osteoclasts, whereas osteoprotegerin blocks this process by functioning as a decoy receptor for RANKL. This novel cytokine system appears to play an important role in the establishment of bone metastases in PCa patients (7).

Osteoprotegerin has recently been found to be overexpressed in bone metastases of these patients (8). This overexpression could indirectly favor osteoblastic reactions by inhibiting osteoclastogenesis and might explain the bone lesions typically seen PCa patients. Because tissue overexpression of proteins often is reflected in blood, osteoprotegerin could be a potential serum marker for diagnosis of bone metastasis.

Using a recently introduced novel osteoprotegerin assay, we performed the present study to evaluate the diagnostic validity of osteoprotegerin as a potential bone metastasis marker in PCa in comparison with sALP and cross-linked N-telopeptides of type I collagen (NTx), which represent osteoblastic and osteoclastic markers, respectively.

The study included 36 male controls (mean age, 51.4 years) with no history of prostate diseases and normal digital rectal examinations, 35 patients with benign prostatic hyperplasia (BPH; mean age, 67.1 year), and 93 patients with carcinoma of the prostate (mean age, 66.2 years). Seventeen of the carcinoma patients had bone metastases, whereas 76 were without bone metastases. All of the men were investigated in the Department of Urology of the Humboldt University or the affiliated outpatient department. We used
all archived sera collected between December 1998 and January 2001 and available as surplus serum (neither thawed nor refrozen) with sufficient volume for additional measurements in this retrospective study. Some (n = 14) cancer patients were on antitumor therapy at the time of sample collection. The study was approved by the Ethical Committee of the Hospital.

The clinical diagnosis of BPH by digital rectal examination, assessment of the International Prostate Symptom Score, and transrectal ultrasonography was histologically confirmed by examination of tissue specimens obtained by sextant biopsy or after transurethral resection. PCa was diagnosed histopathologically by microscopic examination of prostatic specimens obtained at radical prostatectomy or by ultrasound-guided sextant prostate biopsy in all cases. Cancer stage was assigned according the TNM system, and the histologic grade was classified as grade 1, 2, and 3 (9). Patients without bone metastases were subdivided into the groups without (pN0M0; n = 43) and with lymph node metastasis (pN1M0; n = 33). Surgical lymph node staging with histologic examination was performed. Patients without lymph node metastasis had tumor stages pT2 or T3 (n = 24) and pT3 or T3 (n = 19), respectively, with pathologic grades G1 (n = 4), G2 (n = 25), and G3 (n = 14). The patients with lymph node metastasis but without distant metastasis (pN1M0) had tumor stages T2 (n = 10) and T3 (n = 23), respectively, with grades G1 (n = 1), G2 (n = 21), and G3 (n = 11). Bone scintigraphy and, in special cases, x-ray, computerized tomography, or magnetic resonance imaging were applied to diagnose bone metastases in all patients with prostate carcinoma.

In all groups, blood samples were collected in evacuated tubes (Monovette 03.1528; Sarstedt) before any treatment. The interassay imprecision (CV) for NTx (measured as
\[ sALP = \frac{1}{6} \]

and sALP (P = 0.648), but the difference between these two analytes and NTx was significant (P = 0.022).

Fig. 1. Scatter plots of serum osteoprotegerin in controls and patients with BPH and PCa (A) and ROC curves for osteoprotegerin, sALP, and NTx (B).

antibody against osteoprotegerin. After the addition of 100 μL of dilution buffer, 50 μL of sample (or calibrator, control material), and 50 μL of a second biotinylated antibody against osteoprotegerin, the microtiter plate was incubated at 4 °C overnight to form a sandwich complex. The plate was washed five times with a wash buffer to remove unbound and nonspecifically bound substances, and 200 μL of horseradish peroxidase-streptavidin conjugate was added and incubated at room temperature for 1 h. After another wash, 200 μL of tetramethylbenzidine, the substrate for the peroxidase, was added. The reaction was terminated by the addition of 50 μL of ready-to-use stop solution, and the absorbance was immediately measured at 450 nm (HT III Reader; Anthos Labtec). Concentrations were calculated by the four-parameter method.
osteoprotegerin concentrations of 1–3 ng/L and showed a gaussian distribution (Kolmogorov–Smirnov test). The arithmetic mean (SD) was 33.9 (15.9) ng/L. The provisional upper 97.5% reference limit, calculated as the mean + 1.96 SD (14), was 65.1 ng/L. The values showed a tendency to be age dependent ($r_s = 0.323; P = 0.072$) as described by others (12). Serum osteoprotegerin concentrations of 1–3 μg/L in healthy adults (12) and mean values of 230 ng/L in women >65 years have been reported (15). The reasons for these discrepancies are unknown. Differential detection of forms of osteoprotegerin has been assumed, leading to the use of osteoprotegerin ligand as capture protein in another ELISA (16).

The osteoprotegerin concentrations of the controls did not differ from the concentrations found in BPH patients and in PCa patients without bone metastases (Fig. 1A; $P > 0.05$), but were clearly different from the values observed in patients with bone metastases ($P < 0.001$). These results were not attributable to age because there were no age differences between the BPH and PCa patients. In PCa patients, osteoprotegerin showed weak correlations with sALP, NTx, prostate-specific antigen, and tumor stage ($r_s = 0.237, 0.210, 0.237, and 0.222$, respectively; $P < 0.05$), but not with histologic grade of the tumor ($r_s = 0.051; P = 0.641$), with alanine aminotransferase as liver marker ($r_s = -0.077; P = 0.476$), or with serum creatinine as renal marker ($r_s = 0.076; P = 0.474$). Serum sALP, NTx, and osteoprotegerin concentrations did not differ in metastatic patients with and without hormonal therapy ($P = 0.957, 0.957$, and $0.396$, respectively). ROC curves for osteoprotegerin and the two conventional serum analytes sALP and NTx were calculated to compare their abilities to differentiate PCa patients with and without bone metastasis (Fig. 1B). The area under the osteoprotegerin curve was comparable to that of the sALP curve (0.964 vs 0.940; $P = 0.648$), but was significantly higher than the area under the NTx curve (0.782; $P = 0.023$). When we used the above-mentioned cutoff for osteoprotegerin, 15 of the 17 patients with bone metastases were correctly identified [diagnostic sensitivity, 88%; 95% confidence interval (CI), 67–98%], whereas 5 of the 76 patients without bone metastases had increased values (diagnostic specificity, 93%; 95% CI, 87–97%). The corresponding sensitivities and specificities for NTx were 71% (95% CI, 47–88%) and 83% (95% CI, 73–89%) and for sALP were 88% (95% CI, 67–98%) and 95% (95% CI, 90–99%) for sALP based on the 97.5% reference limits of the controls. The calculated specificities may be misleadingly low because scintigraphy, the reference standard, is not 100% sensitive.

Although our study included a limited number of patients, the results suggest that increased serum osteoprotegerin is a marker of bone metastatic spread in PCa patients. As discussed above, the increased serum concentrations could be a reflection of the overexpression of osteoprotegerin in prostatic bone metastases (8). Prospective studies in the follow-up of PCa patients are needed to determine the accuracy and usefulness of this test in early detection of bone metastases and in monitoring during bisphosphonate and other forms of therapy.

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