Evaluation of the LIAISON® Osteocalcin assay in patients with osteoporosis or bone metastases from breast cancer

Introduction

Biochemical markers of bone turnover are increasingly used in patients with metabolic bone diseases. In osteoporosis, significant relationships have been shown between several markers of bone turnover and fracture risk in untreated patients, and with the anti-fracture efficacy of various drugs, especially bisphosphonates.

Markers are also used for the development of new drugs, of new therapeutic schemes or for the validation of new indications. There is also much interest in their use to improve patient compliance to therapy. In cancer patients with bone metastases, significant relationships have been demonstrated between the normalisation of levels of biochemical markers of bone turnover and the occurrence of complications of tumor-induced osteolysis. Their value to tailor bisphosphonate therapy to an individual patient is currently investigated[1,2].

The main biochemical markers of bone turnover that reflect the rates of bone resorption and formation are listed in **Table 1**. Markers of osteoclastogenesis (osteoprotegerin and RANK Ligand) still lack adequate clinical validation.
Different markers may reflect different aspects of bone formation or resorption but significant correlations have been shown between the levels of these markers and the actual rate of bone turnover quantified by calcium balance studies and by histomorphometry techniques[1].

Table 1. Biochemical markers of bone turnover

Bone formation markers	
Blood:	Total Alkaline Phosphatase
	Bone Alkaline Phosphatase
	Osteocalcin
	Procollagen I extension peptides (PICP, PINP)
Bone resorption markers	
Urine:	Hydroxyproline
Urine & Blood:	Collagen pyridinium crosslinks and telopeptides (PYD, DPD, NTx, CTx)
Blood:	ICTP - TRAP

Osteocalcin: a brief overview

Osteocalcin (or Bone-GLA protein, BGP) is the most abundant non-collagenous matrix protein of bone, comprising 1-2% of total bone protein. Osteocalcin, a small protein (49 amino acids) mainly synthesized by mature osteoblasts, remains the most specific marker of osteoblastic activity[3]. Its function has still to be further defined, but it may play a role in bone mineralization and in the regulation of bone turnover, following the binding of its γ-carboxyglutamic acid residues to hydroxyapatite[4].

Osteocalcin is an established biochemical marker of bone formation and of bone turnover[5,6]. In untreated postmenopausal osteoporotic women, osteocalcin levels have been shown to correlate with the risk of an osteoporotic fracture[7,8]. In cancer patients, the value of osteocalcin as a marker of bone metastases deserves further investigation[9]. BGP levels appear to be highly variable in these patients even if they are significantly higher in patients with sclerotic than in patients with lytic metastases[10]. Markers of bone turnover appear to be good predictors of skeletal-related events. The predictive value of the bone resorption marker NTx appears to be superior to the one of the bone isoenzyme of alkaline phosphatase (BAP)[11]. Other markers of bone formation, such as BGP or PINP, could have a greater association with outcome events[12].

Osteocalcin is measured by different types of assays, including high performance liquid chromatography (HPLC), RIA, immunoradiometric assay (IRMA), ELISA and chemiluminescence immunoassay (CLIA)[5,13]. There are several reports showing quite discordant results when BGP is measured by different laboratories[14,15]. Proper sample handling is mandatory. One of the main causes of discordant results is that different assays detect the intact molecule and/or different BGP fragments[16,17]. Intact osteocalcin is unstable due to cleavage between amino acids 43 and 44, resulting in the formation of a big fragment which is more stable[18]. As for the measurement of many analytes, automated assays have many advantages as compared to manual assays that they tend to replace in routine clinical practice. An automated chemiluminescence immunoassay has been shown to be a valuable marker of bone turnover in various metabolic bone diseases[19-21].

a l c i n

Objectives of the study

We have assessed the value of the newly introduced DiaSorin LIAISON® Osteocalcin assay that we have compared to a widely used non radioisotopic fully automated assay with the same specificity, in osteoporotic patients and in patients with bone metastases.

Material and methods

Assays. The DiaSorin LIAISON® Osteocalcin assay and the other commercially available assay (CLIA B) are one-step sandwich chemiluminescence immunoassays (CLIA) that measure intact osteocalcin and the stable big fragment. Both assays were run by the same technician according to manufacturers' recommendations.

Subjects. 5 groups of subjects were studied:

- 76 healthy subjects: 17 premenopausal women (PreMP), 32 postmenopausal women (PostMP) and 27 healthy men
- 20 osteoporotic patients (65±8 yrs) evaluated before specific treatment (OPosis group)
- 19 osteoporotic patients (66±10yrs) under bisphosphonate therapy since at least 6 months (mean duration of therapy: 34 months)
- 16 patients with breast cancer and bone metastases (57±13 yrs) before bisphosphonate treatment (Bone Mets group)
- 18 patients with breast cancer and bone metastases (60±12 yrs) under bisphosphonate therapy since at least 6 months (mean duration of therapy: 16 months)

Results

1. *BGP references values (mean ± SD) and comparison of the pathological groups with healthy subjects*

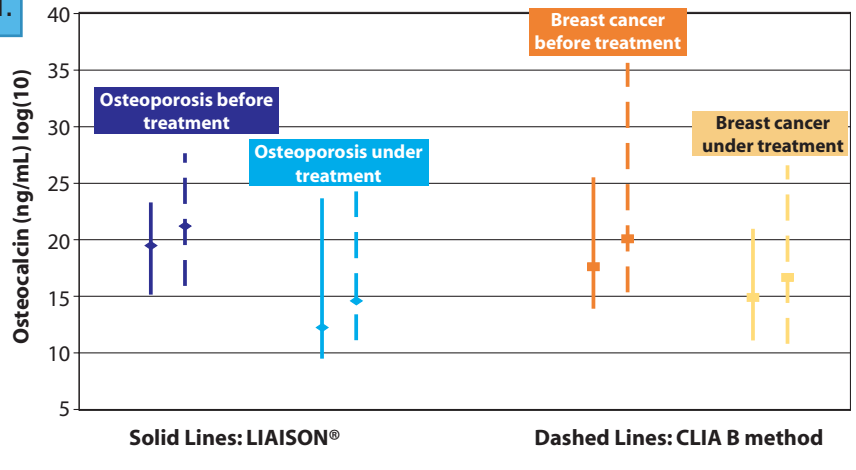
	LIAISON®	CLIA B
Pre-Menopausal women	18.1±3.8	17.1±6.2
Post-Menopausal women	19.5±8.4	17.5±7.3
Men	19.0±6.7	16.8±4.4
Osteoporosis	19.1±5.7	23.7±9.6
Bone Metastases	22.2±16.7	28.7±20.8

BGP values correlated quite well between both assays ($r_s = 0.75$ in healthy subjects, 0.90 in OPosis group and 0.93 in Bone Mets group; all $p < 0.001$). There were significant differences between the healthy group and the pathological groups (Mann-Whitney) with the CLIA B assay (OPosis or Bone Mets vs PostMP only, $p < 0.05$; Oposis or Bone Mets vs Pre + PostMP, $p \leq 0.01$). Scatter of BGP levels was substantial in patients with bone metastases. This is in agreement with our previous findings using other assays[22,23].

2. *Effects of bisphosphonate therapy*

As shown in **Fig. 1**, BGP levels tended to fall after bisphosphonate therapy. Data shown are median values and interquartile ranges for each set of data. We used log(10) transformed data because of non-normal distribution. The decline in BGP levels was not significant in cancer patients with either assay, but reached statistical significance in osteoporotic patients with both assays ($p < 0.05$).

Fig 1.



Summary and conclusions

Osteocalcin levels fell significantly after bisphosphonate therapy in osteoporotic patients with the LIAISON® assay and the other commercially available immunoassay (CLIA B).
In conclusion, this new automated assay on the LIAISON® appears to be as valid as the widely used non radioisotopic fully automated assay (CLIA B) to monitor bone turnover in osteoporotic patients under therapy.
When diagnosis of osteoporosis or bone metastatic involvement are concerned, other examinations are mandatory since bone turnover markers lack sufficient sensitivity and specificity to be diagnostic when used alone.