Sensitivity and specificity of serum cross-linked N-telopeptides of type 1 collagen (NTx) in patients with solid tumors

Sensibilidade e especificidade da dosagem sérica do N-telopeptídeo do colágeno tipo I (NTx) em pacientes com tumores sólidos

Fernando Jablonka¹, Fernanda Schindler², Paula Philbert Lajolo³, Hélio Pinczowski⁴, Fernando Luiz Affonso Fonseca⁵, Antônio Barbieri⁶, Luiz Henrique Massonetto⁷, Claudia Giorgia Bronzatti de Oliveira⁸, Fábio Tadashi Katto⁹, Auro del Giglio¹⁰

ABSTRACT

Objective: Cross-linked N-telopeptides of type 1 collagen (NTx) increase in situations in which bone resorption is increased, such as osteoporosis and bone metastasis. It was evaluated the usefulness of NTx for the diagnosis of bone metastases in patients with solid tumors. Methods: It was conducted a prospective study, at a tertiary public hospital. A quantitative enzyme-linked immunosorbent assay (ELISA) assay of serum NTx levels in 19 subjects without a history of cancer and 62 patients with various solid tumors referred for a bone scan was evaluated. Three specialists read all bone scans. Results: It was not found any significant correlations between serum NTx and age, sex, history of bone pain, tumor type and bone alkaline phosphatase levels. We found a significant correlation between serum NTx and alkaline phosphatase levels ($r^2 = 0.08, p = 0.022$). Serum NTx sensitivity, specificity, positive and negative predictive values and area under the receiver operating characteristic (ROC) curve for the presence of bone metastases were 0.34, 0.92, 0.95, 0.22 and 0.59, respectively. Conclusions: It was concluded that serum levels of NTx are highly sensitive for the diagnosis of bone metastases in patients with solid tumors.

Keywords: Alkaline phosphatase; Bone neoplasms; Predictive value of tests; Collagen type I

RESUMO

Objetivo: Os N-telopeptídeos do colágeno tipo-I (NTx) se elevam quando a reabsorção óssea está aumentada, devido a condições como a osteoporose e a metástase óssea, sendo assim, o objetivo deste estudo foi avaliar a utilidade da dosagem de NTx em uma população heterogênea de pacientes com tumores sólidos para o diagnóstico de metástases ósseas. Métodos: Foi conduzido um estudo prospectivo em um hospital público terciário. Foram analisados 19 pacientes sem história de câncer e 62 pacientes com tumores sólidos de vários tipos, encaminhados para estadiamento e investigação dos sintomas esqueléticos. Utilizou-se a técnica de ELISA (enzyme-linked immunosorbent assay) quantitativa para a dosagem de NTx. Três especialistas leram todas as imagens ósseas, obtidas por medicina nuclear. Resultados: Não foi encontrada correlação entre o NTx, idade, sexo, história de dor óssea, tipo de tumor e níveis de fosfatase alcalina óssea. Foi encontrada correlação significativa entre os níveis de NTx e de...
INTRODUCTION

Bone metastases (BM) are frequent in patients with cancer; they have a potential for complications such as pain, hypercalcemia and pathological fractures. The introduction of bisphosphonates in the clinical practice has contributed to decreased number of these complications.

BM induce increased rates of bone resorption increasing bone formation. Therefore, it is necessary to evaluate the bone resorption markers in patients with solid tumors who present BM. Approximately 90% of bone organic matrix is composed of type 1 collagen, which is a helical protein with cross-linked bonds in its N-terminal and C-terminal. The quantitative assessment of the N-terminal linked to telopeptides of type 1 collagen (NTx) may serve as a marker of increased bone resorption, and it is in present in some diseases, such as osteoporosis and BM diseases. Some studies propose the urine analysis of NTx in patients with solid tumors and BM, with this marker being decreased when they are treated with bisphosphonates. NTx can also be measured in the serum, as a bone resorption biochemical marker with results that are similar to those obtained with the urinary NTx.

Serum measurement may have an advantage compared with the urinary measurement, since in the same sample there is a possibility of investigating other biochemical and hematological markers and also with elimination of the systematic error which can occur due to the collection and storage in the 24-hour urine sample.

Pectasides et al. studied 33 patients with breast cancer and BM and 31 patients with metastatic breast cancer and extra-skeletal metastases, and they showed that patients with BM had significantly higher levels of NTx in the serum when compared with those without BM. In this study, by using a cutoff point of 29.7 nanomoles of bone collagen equivalents per liter (nM BCE) for NTx, the sensitivity and specificity were 87.1 and 45.5%, respectively. In another study, Koizumi et al. showed that the serum NTx was increased in patients with BM, especially in those with widespread bone involvement.

After a search in the literature, it was decided to assess the serum levels of NTx in samples of patients with solid tumors who underwent bone scintigraphy either for staging or for assessment of bone pain. This group of patients was compared with another group free of oncological and bone disease in regard to their serum levels of NTx. Patients with BM included in this study had significantly higher levels of NTx than patients without BM and normal controls.

OBJECTIVE

To describe the operational features of serum NTx for diagnosis of BM in patients with solid tumors and the correlation with the levels of bone and total alkaline phosphatase.

METHODS

In a prospective and non-consecutive manner, it was included 62 patients seen at Hospital Estadual Mário Covas da Fundação e Faculdade de Medicina do ABC, who signed the informed consent term after approval by the Institutional Ethics Committee. These patients were adults, had been diagnosed with solid tumors and did not know the diagnosis of BM because they still had not undergone imaging examinations for that purpose. Patients treated with bisphosphonates were excluded, but the patients who received previous systemic treatments or radiotherapy were not excluded. This sample composed of 62 patients who had already been used in another study to assess the operational features of the serum levels of bone alkaline phosphatase and total alkaline phosphatase. A total of 19 individuals without history of cancer, osteoporosis or recent fractures were also included. These normal individuals were used as controls for the serum study of NTx.

Two to three hours after injection of 1.110 MBq 99mTc-MDP (Instituto de Pesquisas Energéticas e Nucleares – IPEN, São Paulo, Brazil), the patients underwent the scan examination. The images were obtained with the help of a two-head camera (Forte – ADAC Laboratories, Milpitas, CA), equipped with a low-power high resolution parallel view, at 30 cm/min, 128 × 128 × 16 matrix in size. The images were obtained in a computer dedicated to nuclear medicine (Pegasys – ADAC). Power was supplied by a 20% discrimination of the window centered at 140 keV photopeak 99mTc. Two specialists examined the bone scintigraphies and a third skilled specialist was a consultant in cases of disagreements between the first two specialists. Bone scintigraphies were interpreted based on previous experiences, and they were performed by three
physicians specialized in nuclear medicine classifying the results as high, intermediate or low likelihood of BM.

Radiography, tomography and magnetic resonance imaging were used in cases of suspected lesions. The finding was considered a metastasis when during the follow-up period the following were observed: fracture, bone pain, necessity to start bisphosphonates treatment, an image or a test showing a bone lesion.

Concurrently with bone scintigraphy, it was also measured the serum levels of ionized and total calcium, phosphorus, magnesium, total proteins, albumin, total alkaline phosphatase, bone alkaline phosphatase, and NTx. Total alkaline phosphatase was measured by quantitative method of inactivation with the substrate p-nitrophenyl phosphate (pNPP); the reference values used were those measurements between 100 and 290 U/l (Bayer, São Paulo, Brazil). Bone alkaline phosphatase was measured through the immuno-enzymatic method with the aid of Alkaphase-B Kit (Quidel/Metra Biosystems, Mountain View, CA). NTx levels were assessed by means of enzyme-linked immunosorbent assay (ELISA) using the kit Osteomark NTx®; this serum test is a competitive assay (Inc., Princeton, NJ), according to Kanakis et al.(3). The reference values recommended by the manufacturer were considered; for women, they ranged from 6.2 to 19 nanomoles of bone collagen equivalent per liter (nM BCE) and for men the reference values were between 5.4 and 24.2 nM BCE.

The area under the curve for the ROC (receiver operating characteristic) was calculated; through this evaluation, it was also calculated the sensitivity, specificity and positive and negative predictive values. The associations between categorical variables were assessed by Fisher and the $\chi^2$ test and the associations between continuous variables with normal distribution and categorical variables by means of the analysis of covariance (ANOVA). The regression analysis was used to evaluate the correlation between the continuous variables. The statistical package NCCS 2000 (http://www.ncss.com/) was used for all statistical calculations.

RESULTS

A total of 62 patients were enrolled, and their demographic and clinical characteristics are shown in Table 1. Twenty-seven patients had a high likelihood of presenting BM in the initial bone scintigraphy, but after the review graph performed six months after the examination, it was seen that in three of these patients, their physicians had not considered them as having BM. Indeed, during a further review of those patients’ medical records, there was no evidence of clinical or skeletal symptoms.

![ROC curve for serum NTX](image)

It was considered that, in light of the above-mentioned follow-up, these three patients did not present BM. None of the other patients who had an intermediate or low probability classification according to bone scintigraphy showed any evidence of having BM with any of the above-mentioned criteria during their six-month follow-up after scintigraphy, thus being considered as not having BM. A total of 19 control individuals (6 males and 13 females), with a mean age of 59.10 ± 3.75 years were also included.

When taking into account only patients with cancer, a significant correlation between the serum levels of NTx and age, sex, history of bone pain, type of tumor or levels of bone alkaline phosphatase was observed. However, it was found a statistically significant correlation between serum NTx and alkaline phosphatase (ALP) levels ($r^2 = 0.08$, $p = 0.022$).

Using the cutoff values suggested by the manufacturer, the serum levels of NTx, sensitivity, specificity, positive and negative predictive values and ROC AUC for the presence of BM were 0.95, 0.22, 0.34, 0.92 and 0.59, respectively (Figure 1).

![Table 1. Clinical and demographic characteristics of 62 patients enrolled in this study](image)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Presence (BM) $n = 24$</th>
<th>Absence (BM) $n = 38$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.41 ± 2.48</td>
<td>57.44 ± 1.97</td>
<td>0.065*</td>
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<tr>
<td>Sex</td>
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<td>Male</td>
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<tr>
<td>Breast cancer</td>
<td>12</td>
<td>23</td>
<td>0.55*</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>15</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Other</td>
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<td>7</td>
<td></td>
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<tr>
<td>Reason for performing bone scintigraphy</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>7</td>
<td>6</td>
<td>0.20*</td>
</tr>
<tr>
<td>Staging</td>
<td>17</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

* no significant correlation was found between the serum NTx levels and age, sex, history of bone pain and type of tumor
DISCUSSION

BM are frequent in cancers for which efficient prevention already exists, such as periodical prophylaxis and administration of bisphosphonates\(^\text{[1,2,9]}\). However, early detection of BM is necessary to avoid its deleterious effects in the patient's clinical status\(^\text{[3,10]}\).

BM increases bone formation and resorption; therefore, it was decided to test the functioning of serum NTx in samples of patients with solid tumors. Curiously, its high 95% sensitivity in this setting assures its use as a good test for tracking the presence of BM in patients with solid tumors. However, the low specificity of the test may require the use of other serum markers with higher specificity such as alkaline phosphatase and bone alkaline phosphatase\(^\text{[11-12]}\), in order to decrease the rate of false positive tests of serum NTx. The specificity of serum alkaline phosphatase is 100%, but the sensitivity is much lower, of only 0.52; the sensitivity of bone alkaline phosphatase was 0.86 and its specificity was 0.69 in the same patients. Therefore, in this experience, NTx seems to be more sensitive than alkaline phosphatase and bone alkaline phosphatase to detect metastases in a population of patients with several solid tumors.

In regard to sensitivity and specificity, the present results are different from those reported by Pectasides et al.\(^\text{[12]}\), who, using a cutoff point of 29.7 nM BCE for NTx, reported a specificity of 87.1% and sensitivity of 45.5%. These authors, however, restricted their analyses to patients with breast cancer and used a higher cutoff value of serum NTx than that recommended by the manufacturer and applied in our study. However, if we had used the cutoff point of 29.7 nM BCE, as suggested by these authors, the sensitivity of serum NTx for detection of BM would fall to 0.75 and its specificity would increase to 0.50. Likewise, with this higher cutoff point, the negative predictive value would fall to 0.82 and ROC AUC would slightly increase from 0.59 to 0.62.

Several authors showed that increased levels of serum or urinary NTx may decrease after treatment with bisphosphonates as a sign of a response of non-absorption of treatment\(^\text{[4,7-8]}\). Therefore, along with its high sensitivity in the detection of patients with BM before treating with bisphosphonates, serum evaluation of NTx was also helpful during the follow-up of patients with BM who were treated with these agents.

Therefore, if it was obtained the measurement of serum NTx at presentation of all patients with solid tumors or if patients have a normal level, they are unlikely to have BM. Additionally, if BM are identified in the context of a normal serum NTx, the benefits of bisphosphonates may not be very obvious\(^\text{[4]}\). Based on our data, we believe that asymptomatic patients with normal serum levels of NTx may be spared from bone scintigraphy.

However, if serum levels of NTx are abnormal, it would be convenient to have patients undergo bone scintigraphy – especially if their simultaneous levels of alkaline phosphatase are also abnormal – and to start administration of bisphosphonates if one or more BM are identified. It was recommended a careful follow-up of patients with increased serum levels of NTx and a normal scintigraphy, since the patients may have a higher chance of developing BM and adverse bone events in the future\(^\text{[4]}\). Patients with high serum levels of NTx and no BM may also be at risk of losing the bone mineral density and should undergo periodical densitometry. Osteoporosis, when present, may also justify the use of bisphosphonates.

CONCLUSIONS

It was concluded that the high sensitivity of serum NTx in this context deserves a more comprehensive analysis about its value for tracking patients with solid tumors as to the presence of BM, especially if it is associated with higher specificity serum tests, such as bone alkaline phosphatase and total alkaline phosphatase measurements. New studies should also be conducted to confirm our results and to evaluate them in regard to the diagnosis of BM by means of new tracking technologies, such as PET/CT.
ACKNOWLEDGEMENTS

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REFERENCES