Value of Procollagen Type 1 Amino-terminal Propeptide in Patients with Renal Cell Carcinoma

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Abstract. Background: The aim of the study was to investigate whether the bone turnover marker procollagen type I amino-terminal propeptide (P1NP) could be useful for the early detection of bone metastases in patients with renal cell carcinoma (RCC) and if chemotherapy influences P1NP concentrations in patients with bone metastases. Patients and Methods: Serum samples of 36 patients were analyzed using a specific immunoassay. The patients were divided into three groups: 24 patients without metastatic spread, 6 patients with untreated bone metastases and 6 patients who had received sorafenib. Results: The P1NP concentration was significantly higher (p≤0.001) in the patients with bone metastases (median: 396.10 ng/ml) than in those without bone involvement (median: 35.53 ng/ml). The patients treated with sorafenib showed levels within the normal range (median: 28.96 ng/ml). Conclusion: P1NP is a significant diagnostic marker for the development of bone metastases in patients with RCC and could help to evaluate the progress of chemotherapy.

Renal cell carcinoma (RCC) is a very important urological cancer. In 2007, there were 51,190 new cases of RCC and 12,890 deaths in the United States (1). The musculoskeletal system is one of the most common sites of metastatic spread in patients with renal cell carcinoma apart from the lung, liver and brain (2). Thus, examining these patients for bone metastases is an important aspect of patient care. The evaluation of metastatic bone disease is also crucial for the primary cancer staging because the appearance of bone metastases influences the short-term prognosis and increases morbidity and mortality. In clinical routine, bone involvement is usually detected by X-ray, computed tomography and by means of bone scintigraphy, but results are rather disappointing in patients with RCC (3). As bone metastases are associated with an increase in bone metabolism, new techniques try to identify malignant bone turnover by means of special tumour markers. As type I collagen is a major structural protein in bone, its precursor molecule procollagen type I amino-terminal propeptide (P1NP) effectively reflects malignant changes in bone formation (4). Until now, P1NP has given proof of being useful for the detection of bone metastases in patients suffering from prostate carcinoma (5, 6) and breast cancer (7). In these studies, bone metastases were paralleled by a significant increase in serum P1NP concentrations.

The aim of the present study was to examine whether P1NP might be a suitable marker for the early detection of bone metastases in RCC. Additionally, whether or not chemotherapy, improving the patient’s condition and extending the progression-free survival, may lead to a normalization in serum P1NP levels, was investigated.

Patients and Methods

Our unblinded study included 36 patients with histologically confirmed RCC, including 26 patients who were treated in the Hospital of the Johann Wolfgang Goethe University Frankfurt, 7 patients from the Großhadern Hospital of the Ludwig Maximilian University München, two patients from the St. Markus Hospital, Frankfurt, and one patient from the Town Hospital Hanau. For the purposes of this study, they were divided into 3 groups: the first group contained 24 patients with RCC without bone metastases (TxM (0)), the second included 6 patients with skeletal metastases who had received neither radiation nor chemotherapy (TxM (s)) and the third consisted of 6 patients with bone metastases who had been treated with sorafenib in 2006 (TxM (s)+S). Table I presents the demographic and clinical characteristics of the three study groups. For clinical staging, the patients were classified in accordance with the TNM staging system, using ultrasonography, computed
tomography or magnetic resonance imaging. To determine the presence of bone metastases bone scintigraphy was performed. Exclusion criteria were bone fractures near to the time of blood sampling and manifest osteoporosis. Two patients with RCC had to be excluded from the study because they additionally suffered from hyperparathyroidism. They manifested excessively high values of P1NP (657.2 ng/ml and 1084.2 ng/ml, respectively) without showing any sign of bone metastases. No patient was taking drugs known to affect bone metabolism.

To perform the P1NP measurements, serum samples were collected from the 36 patients with RCC between January 2006 and July 2007. Thirty of the samples were taken prior to chemotherapy and radiation. To study the effect of chemotherapy on the P1NP levels in patients with bone metastases, samples were collected from six of the patients after they completed treatment with sorafenib. The sera were separated from blood by centrifugation within 1 h after sample collection and then frozen and stored at –80°C until P1NP analysis. Serum P1NP was measured with the electrochemiluminescence immunoassay ECLIA using an Elecsys 1010 analyzer (Roche Co., Mannheim, Germany). This method is based on a sandwich principle, using streptavidin-coated microparticles, biotinylated monoclonal anti-P1NP antibodies and monoclonal anti-P1NP antibodies labeled with a ruthenium complex. Alkaline phosphatase (AP), another marker of bone formation, was also measured in order to compare its diagnostic effectiveness with P1NP.

The values are shown as median and range. Spearman’s coefficient of linear correlation between markers of bone metabolism (P1NP and AP) was calculated. Sensitivity, specificity and positive and negative predictive values of both markers of bone formation were also calculated. All the statistical calculations were performed with SPSS® 11.0, SPSS Inc. 2001 and Microsoft® Office Excel 2003.

**Results**

Table II and Figure 1 show the values of P1NP in the patients with and without bone metastases. In this study, the P1NP cut-off was 60 ng/ml. The P1NP level was significantly higher in the patients with bone metastases (median: 396.10 ng/ml) than in those without bone involvement (p≤0.001), who had P1NP concentrations within the normal range (median: 35.53 ng/ml). Additionally, Table II and Figure 1 illustrate an obvious influence of chemotherapy on serum P1NP levels. The patients with bone metastases who had received chemotherapy had significantly lower P1NP values than the patients who had not been treated with chemotherapy or radiation (p≤0.001). All the patients subjected to previous chemotherapy presented levels within the normal range (median: 28.96 ng/ml), except one patient, whose P1NP value just slightly exceeded the upper limit of our study protocol (61.06 ng/ml). Furthermore, the median serum P1NP of the patients treated with sorafenib was even lower than in the patients without any sign of bone metastases. This may be partly due to the fact that three patients in the group without bone metastases had P1NP values higher than the cut-off (60 ng/ml). It is interesting to note that two of these patients were postmenopausal women (one aged 74 years, P1NP 64.59 ng/ml; and one aged 60 years, P1NP 67.66 ng/ml) who had not been treated with hormone replacement therapy and thus were allowed higher values of P1NP (≤76.31 ng/ml according to the reference values for the ECLIA). The third patient was suffering from a B-cell lymphoma apart from his renal cell carcinoma (P1NP 82.34 ng/ml).

In this study, the cut-off value for AP for male patients was 129 U/l and for female patients was 104 U/l. Table II shows that the AP was significantly higher in patients with untreated bone metastases (median: 358 U/l) than in the study group without malignant changes in bone metabolism (median: 66 U/l) (p≤0.001). The patients who had been

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**Table I. Characteristics of the study group.**

<table>
<thead>
<tr>
<th>TxM (0)</th>
<th>TxM (s)</th>
<th>TxM (s) + S</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>60.6</td>
<td>68.8</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>T2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>T3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>T4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TxM (0): no bone metastases; TxM (s): with bone metastases, no radiation or chemotherapy; TxM (s)+S: with bone metastases, treated with sorafenib.
treated with sorafenib had median AP levels within the normal range (median: 103.0 U/l), thus being significantly lower than the AP concentrations in patients with untreated bone metastases ($p \leq 0.01$).

A positive correlation was shown between the two markers of bone formation (correlation coefficient 0.474; $p \leq 0.01$). Table III shows that P1NP was superior to AP in sensitivity, specificity and positive and negative predictive value.

**Discussion**

Jung et al. have found that several important markers of bone turnover, such as the bone-specific AP, cross-linked N-terminal and tartrate-resistant acid phosphatase isoenzyme 5b, were not successful in detecting bone metastases in patients with RCC (8). We are the first to focus on P1NP, another marker for increased bone formation (9). In our examination, 100% of the patients with untreated bone metastases showed significantly elevated P1NP levels, thus offering the possibility of a reliable diagnosis of bone involvement in patients with metastatic RCC. In clinical routine, AP is often used as an indicator for possible bone metastases in patients with other malignancies, for example prostate carcinoma (10). This study confirmed the findings of Kriteman et al. who have already proved that AP is an unreliable marker for bone metastases in patients with RCC (11). The present study suggested that P1NP was an effective diagnostic marker for the early detection of bone metastases, although future prospective research with larger cohorts is necessary to substantiate this approach.

Sorafenib is a new drug which works by inhibiting a Raf kinase and thus reducing tumour angiogenesis and additionally blocks the vascular endothelial-derived growth factor receptor and platelet-derived growth factor receptor, thus reducing tumour angiogenesis (12, 13). Although RCC is generally considered to be a chemotherapy and radiotherapy-resistant disease (14, 15), a significant decrease of P1NP values was found in the sorafenib-treated group in the present study. All 6 patients who received sorafenib presented serum concentrations within the normal range of P1NP, indicating a decline in malignant skeletal processes.

This corresponds to the findings of Sciarra et al., who observed a normalization of metastatic bone involvement under treatment with sorafenib with the help of computed tomography (16). P1NP may therefore be useful for monitoring the response to chemotherapy in patients with bone metastases.

**Conclusion**

P1NP is a very useful marker for the detection of bone metastases in patients with RCC, since bone involvement leads to a significant increase of serum P1NP concentrations.

Furthermore, P1NP measurement appears to be helpful for monitoring the success of chemotherapy in patients with bone metastases. The low values of P1NP after chemotherapy reflect the positive influence of sorafenib on patients with metastatic RCC.

**References**


