Pharmacology and Chemical Biology

Original Article

Bone Sialoprotein Antibody to Estimate Risk of Bone Metastasis in Prostate Cancer Patients

Christos Philippou[1], Dirk Theegarten[2], Simon Gloger[3], Burkhard Ubrig[3], Bitterlich Norman[4], Naseva Emilia[5], Andreas Wiedemann[6], Hans-Jörg Sommerfeld[7], Abdulla Haji[8], Stathis Philippou[1]

[1]Institute of Pathology, Augusta-Hospital, Bochum Germany
[2]Institute of Pathology, University Duisburg-Essen, Medical Faculty, Essen Germany
[3]Clinic of Urology, Augusta-Kranken-Anstalt, Witten/Herdecke University, Bochum Germany
[4]Freelance statistician, Chemnitz, Germany
[5]Biostatistician, Hospital TsaritsaYoanna, Medical UniversitySofia, Bulgaria
[6]Clinic of Urology, Evangelical Hospital Witten, Department of Geriatry, Medical Faculty, University Witten/Herdecke, Germany
[7]Clinic of Urology, Marien Hospital, Marl Germany
[8]Institute of Infectiology, Medical Faculty of the University Duisburg-Essen, Essen, Germany

Correspondence

Christos Philippou

Institute of Pathology, Augusta-Hospital, Bochum Germany
Tel: +49 234 5172250
Fax.: +49 234 9585604
E-mail: stathis.philippou@rub.de

- Received Date: 05 Mar 2024
- Accepted Date: 30 Mar 2024
- Publication Date: 02 Apr 2024

Keywords:

antibody; Bone sialoprotein; immunohistochemistry; prostate cancer

Abbreviations

BSP: Bone sialoprotein; ISUP : International Society of Urological Pathology; MAX/MIN: Maximum/Minimun; Mm: Group with metastasis, Mo: Group without metastasis, MW: Mean value, PSA: Prostate specific antigen, Q1/Q3: First/ThirdQuartile, SD: Standard deviation, pU: p-value of Mann-Whitney-U, pSR: p-value of Spearman-Rho, r: Correlation coefficient, Z: Test size of Mann-Whitney-U

Abstract

Background: Osseous metastasis is a typical site of metastasis in patients with prostate cancer.
Objective: To evaluate the role of bone sialoprotein (BSP) expression in prostate cancer patients with bone metastasis.
Design, setting and participants: Immunohistochemistry and quantification were used to evaluate the BSP expression in fine needle punctures of patients with prostate cancer. Data of patients were followed up for 7 to 9 years for detection of bone metastasis.
Results and limitations: 12.5% (84/673) of patients developed osseous metastasis. Of all patients, 20.8% were BSP-negative (0% expression) and 22.2% presented high values of more than 40%. Patients with bone metastasis had higher BSP expression than patients without bone metastasis (55.5 ± 19.7% vs. 25.7 ± 24.9%;p< 0.001). 82.9% of patients with metastasis had a BSP expression of at least 40%, whereas no metastatic patient had a BSP value of less than 20%. For BSP as a single parameter with a cut-off value of 50%, the overall sensitivity was 50% with a specificity of 81.6%.
Conclusions: BSP could be an indicator for the development of bone metastasis. Despite a high specificity, the sensitivity is insufficient for the integration of BSP as a single parameter into clinical routine. Further parameters must be added to the calculation to increase the sensitivity.
Patient summary: A typical site for metastasis of patients with prostate cancer is the bone. In this study, we assessed a marker (bone sialoprotein) in prostatic tissue of patients with prostate cancer, which could be an indicator for later development of bone metastasis.

Introduction

As life expectancy increases in Western industrialized nations, the incidence of all malignant neoplastic diseases is rising [1].The most common neoplasm affecting the male population is prostate carcinoma. Prostate carcinoma accounts for every 4th newly diagnosed malignancy in males and, in 2020, it was responsible for 65.820 newly diagnosed cases and 15.403 deaths just in Germany [2]. The numbers of new cases for the same year worldwide were 1.41 million and 375,304 reported deaths [3]. An important prognostic factor for patients with newly diagnosed prostate cancer is whether the disease is metastatic or not. By far the most common site of metastasis formation in prostate carcinoma patients is bone tissue. Bone tissue is the site of metastasis in up to 80% of patients with an advanced-stage prostate carcinoma [4]. The bone sialoprotein (BSP) is suspected to have a central role in the pathomechanism of bone metastasis formation. BSP was first discovered in studies involving bovine cortical bone [5]. BSP is now known to belong to the same protein family as osteonectin and osteopontin and builds 12% of the total non-collagenic extracellular protein in bone tissue [6]. It is also suspected to have a physiologic role in apatite crystal depletion. Thus, its highest expression physiologically is found in cells which are involved in mineralization processes such as osteoclasts, osteoblasts, osteocytes, chondrocytes, and trophoblasts [7,8].

Since the early 1990s, the role of BSP in the pathomechanism of bone metastasis formation

Citation: Philippou C, Theegarten D, Gloger S. Bone Sialoprotein Antibody to Estimate Risk of Bone Metastasis in Prostate Cancer Patients. Pharmacol Chem Bio. 2024;1(1):01.

has been a focus of scientific research.

It has been found that BSP is expressed by multiple tumours that primarily tend to form metastasis in bone tissue including breast cancer [9], lung cancer [10], and prostate [11] and cervical carcinomas [12].

Recent studies [13] with interleukin (IL) 8 manipulated cell cultures for the upregulation of BSP synthesis show an increased cell adherence to bone in the manipulated culture in comparison to cells that are not treated with IL8.

Differences in the protein structure of physiologically formed BSP and that formed by carcinoma cells make it possible to bind and inactivate it using tomour BSP specific antibodies. Studies in animal models have shown that the simultaneous administration of tumour cells and tumour-specific antibodies against BSP leads to a reduction in the occurrence of bone metastases [14].

Therefore, the research goal of this study is to investigate whether BSP expression in prostate carcinoma has a predictive value for the development of bone metastasis and whether a BSP antibody can be integrated into histopathological routine diagnostics.

Material and methods

The patient collective consists of 1201 individuals who have been diagnosed with prostate cancer after fine needle puncture, in urology unit, during the period 2011–2013 at the Institute of Pathology, Augusta-Hospital, Bochum, Germany. Data and information about osseous metastasis were obtained from 673 of the patients during a 7–9 year follow-up.

The vote of the institutional ethics committee (Medical Faculty of the University Duisburg-Essen, 20-9548-BO) had been obtained before the start of the study.

Three antibodies were tested for reliability and stability. Only one antibody, namely Linaris (Linaris Biological Products, Dossenheim, Germany) in 1:1500 dilution showed sufficient stability in our positive control on placenta and bone tissue. Antibody reactions with the other two antibodies were repeated multiple times but results remained unsatisfactory even with heat and enzyme pretreatment.

In the following study, immunehistochemistry with Linaris (1:1500) was used in the whole patientcollective. The reaction was performed manually by a laboratory assistant and automated with a benchmark Ventana device.

The quantification was done under a light microscope by two observers. Immunohistochemistry and quantification were repeated for the metastatic patients to ensure the accuracy of the BSP value.

Any reaction of the tumour cells was noted as positive. All tumour cells in the tissue cylinder were examined and the ratio of positive/all tumour cells in % was calculated.

Results

12.5% (84/673) of patients with prostate cancer had developed bone metastasis. The metastatic patients were on average older than the non-metastatic patients (p=0.003, see Table 1). Furthermore, there was a statistically significant correlation between the average age and BSP expression in the non-metastatic group (r = 0.088; pSR = 0.037) whereas in the metastatic group, there was no correlation (r=0.162; psr= 0.147, see Table 1).

The average BSP in the metastatic group was 55.5%, while in the non-metastatic group it was 25.7%. This difference in BSP expression between the metastatic and non-metastatic groups was statistically significant (Z = −9.429; pU< 0.001; see Table 2).

Table 1. *Statistically significant difference in age between metastatic (84) and non-metastatic groups (n = 589, Z = −2, 0.933; pU= 0.003).*

Statistical parameter Age [years]								
Group	**N**	**Mean**	**SD**	**Min**	**Q1**	**Median**	**Q3**	**Max**
Metastatic	84	72.5	7.6	51	67.0	74.0	78.0	89
Non metastatic	589	69.8	8.0	46	65.0	71.0	75.0	92

Table 2. *Statistical parameters of bone sialoprotein expression (BSP) (Z = −9.429; pU< 0.001)*

Statistical parameter BSP [%]								
Group	**N**	**Mean**	**SD**	**Min**	**Q1**	**Median**	**Q3**	**Max**
Metastatic	82	55.5	19.7	20	40.0	55.0	70.0	95
Non metastatic	567	25.7	24.9	0	1.0	20.0	40.0	100

Table 3. *Statistical parameters for BSP expression of ≥20% (Z = −5.054; pU< 0.001)*

Statistical Parameter BSP, minimum 20% [%]								
Group	**N**	**Mean**	**SD**	**Min**	**Q1**	**Median**	**Q3**	**Max**
Metastatic	82	55.5	19.7	20	40.0	55.0	70.0	95
Non metastatic	319	42.8	20.3	20	25.0	40.0	60.0	100

Table 4. *BSP as a single parameter. Sensitivity and Specifity using logistic regression.*

BSP	N = 82	50.0%	N = 567	81.6%	50.7%	81.7%	70.0	95

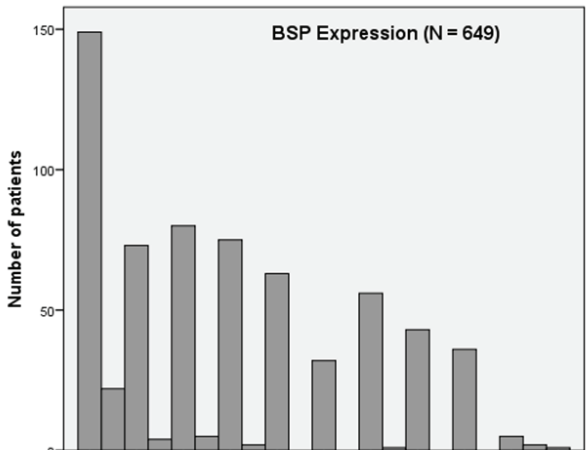

Figure 1. *BSP expression in the patient collective (649).*

Although a BSP value of at least 20% was measured in all patients in the metastatic group, there were 135 patients (135 of 567; 23.8%) in the non-metastatic group with a BSP value of 0% and a total of 248 patients (248 of 567; 43.7%) with a BSP value of less than 20% (Figure 1).

In the patient collective, 20.8% were BSP-negative (0% expression) and 22.2% presented high values (>40% expression, Figure 1).

Overall, 82.9% of patients with metastasis had a BSP expression of at least 40% (Figure 2), whereas no metastatic patient had a BSP value of less than 20% (Figure 3) and none were BSP-negative.

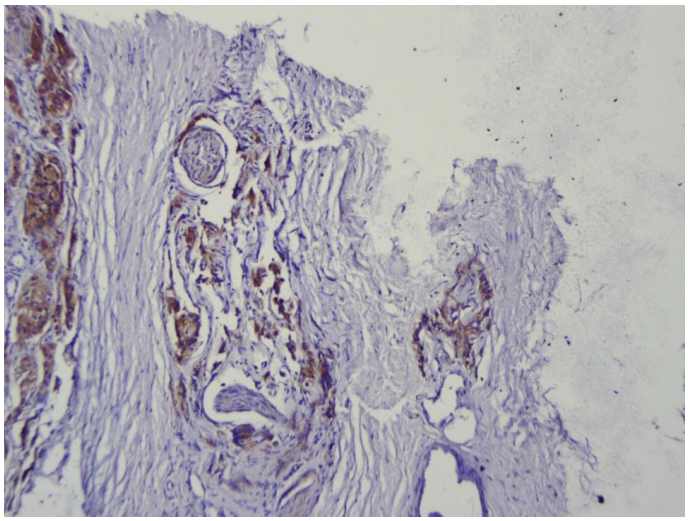

Figure 2. *Strong BSP expression of 90% in poorly differentiated prostate carcinoma, (G3, ISUP 5), with capsular and perineural invasion. Immunohistochemistry: BSP, DAB, original magnification: 100×.*

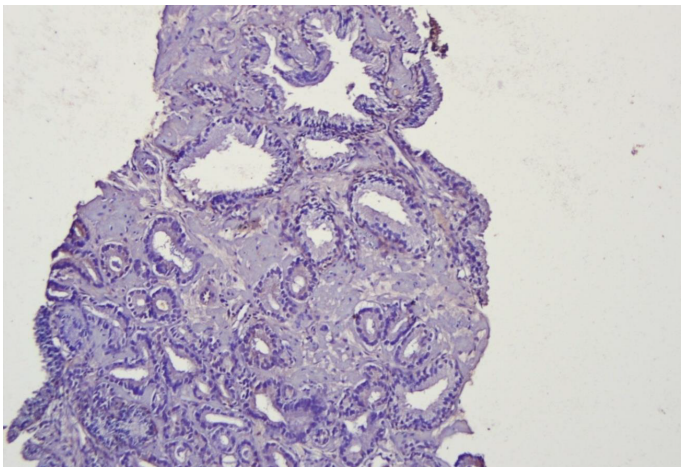

Figure 4. *Low BSP expression (20%) in a moderate differentiated acinar prostate carcinoma (G2, ISUP 2). Immunohistochemistry: BSP, DAB, original magnification: 100×..*

To avoid bias, the calculations have been repeated excluding all patients with BSP of less than 20% in the non-metastatic group. The results have been confirmed. The difference in BSP expression remained significant ($Z = -5.054$; $pU < 0.001$, see Table 3).

Using BSP as a single parameter with a cut-off value of 50% the overall sensitivity was 50% (correct prediction for 41/82 metastatic patients) with a specificity of 81.6% (correct prediction for 464/567 non-metastatic patients) (Table 4).

Discussion

According to the literature, BSP plays a key role in the pathomechanism of osseous metastasis.

BSP expression has been demonstrated in various primary tumours with osteotropism. Several studies have demonstrated the expression of BSP in malignancies of the lung [15], the cervix uteri [12], the breast [9], and the prostate [11], but, to the best of our knowledge, no study could describe a correlation between BSP expression and osseous metastasis in prostate cancer patients.

As in our work, the immunohistochemical examination of paraffin-embedded material has been used in comparable studies on BSP expression in tumour tissue by Waltregny et al. [11], Bellahcene et al. [15], and Detry et al. [12].

Furthermore, Liu et al. [16] published a study in which cell cultures of prostate carcinomas were treated with IL8-specific antibodies. It is assumed that IL8 increases the cell adherence of prostate carcinoma cells to the bone tissue by upregulating BSP expression. Consequently, the authors describe a significant reduction in cell adherence to the bone tissue compared to the non-treated cell culture.

Most interesting, however, is the work of Waltregny et al. [11], in which the authors examined the histological material

of 180 patients with localised prostate carcinoma for BSP expression and clinical and biochemical data (Gleason score, PSA, capsular rupture). In 78.9% of cases, they were able to detect immunohistochemical expression of BSP in degenerated prostate tissue. The authors concluded that increased BSP expression in the primary tumour is associated with an increased risk of osseous metastases in the further course of the disease, as well as with tumour progression. Nevertheless, they were unable to determine a cut-off value.

In this study, we were able to detect BSP expression in 77.6% of our patient collective. However, the patient collective of Waltregny et al.[11] with 180 examined cases was significantly smaller than ours with 673 cases. Thus, a small deviation of BSP-positive tissue in both studies emphasises the accurate performance of the staining in both studies and a similar sensitivity of both antibodies used.

In our study, none of the patients with metastatic prostate carcinomas had BSP-negative primary tumours. This is in contrast to the work of Carlinfante et al. [17], who found that 11% of the primary tumours in patients with metastatic prostate carcinomas showed no BSP expression, but bone metastases occurred in 100%. However, the patient cohort in the study by Carlinfante et al. [17] only included 9 patients with bone metastasised prostate cancer, compared to 84 patients in our study. A failure of the antibody in one of the stains used by Carlinfante et al. [17] can therefore not be ruled out.

Conclusion

BSP can be an indicator for the development of bone metastasis. Although showing a high specificity of 81.6% the sensitivity of 50% is insufficient for the integration of BSP as a single parameter into clinical routine. Patients with a BSP value of less than 20% are not at risk for bone metastasis development, whereas patients with values of more than 40% are at increased risk for the formation of bone metastasis.

Further parameters could be added to the calculation to increase the sensitivity.

Conflicts of interest

The author declares that no conflicts of interest are present.

Acknowledgment

The authors thank all participating urologists for the sharing of clinical data.

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