

# Detection of bone marrow metastases in prostate cancer: Role of trephine biopsy and immunohistochemistry

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## ABSTRACT

**Context:** Bone marrow (BM) metastases are an integral part of tumor dissemination in many malignancies. Prostate carcinoma, which has high tendency to metastasize to bone, shown to have affinity for endosteal niche as well as tendency to compete with hematopoietic cells to home in BM. This marrow dissemination can be confidently proved by histopathological examination of BM. **Aims:** In this study, we are trying to detect the presence of metastases and micrometastases in BM of prostate carcinoma patients with the help of immunohistochemical markers prostate-specific antigen (PSA) and prostate-specific acid phosphatase and correlate the findings with American Joint Committee on Cancer Tumor, Node and Metastasis 7<sup>th</sup> (2010) classification, serum PSA, and biopsy Gleason's score. **Materials and Methods:** We performed BM examination and hematological workup of 11 known prostate carcinoma patients including metastatic cases also, during our study period of 1 year. The BM biopsy sections and clot sections were used to carry out immunohistochemistry. The data were analyzed by using SPSS (Statistical Package for Social Sciences) version 15.0 statistical analysis software. **Results:** We found that two patients were positive for metastases in BM out of the 11. Both of these patients already had metastases to other site with very high serum PSA levels. Anemia was common hematological alteration in both of them and one of them showed increased osteoblasts in the aspirate film. **Conclusion:** Taking into account our small sample size and short study duration, we conclude that further large sized future studies with long-term follow-up in to this BM dissemination by prostate cancer cells could open new horizons to understand the biology of metastasis of this common malignancy and also provide more effective therapeutic options as well as prognostic implications in these patients.

**Key words:** Bone marrow metastasis, immunohistochemistry, prostate carcinoma, trephine biopsy

## INTRODUCTION

Prostate carcinoma is one of the most frequent malignancies of males all over the world. Distant lymph nodes including paraaortic, common iliac, inguinal, and supraclavicular involvement are classified as pM<sub>1a</sub>, while bone metastases are assigned as stage pM<sub>1b</sub> in current TNM classification.<sup>[1]</sup> The term "Micrometastases" was introduced to describe isolated tumor cells which are not detected by conventional staging methods. Micrometastases are microscopic deposits (less than 2 mm) of malignant cells that are

segregated spatially from primary tumor and depend on neoangiogenesis to propagate.<sup>[4]</sup>

The bone marrow (BM) involvement by disseminated tumor cells has poor prognosis in cases of prostate carcinoma as well as in other malignancies. Magnetic resonance imaging (MRI) can give some clues on involvement of BM involvement; the definitive diagnosis depends on histopathological examination of BM biopsies. As stated in 2011 study,<sup>[2]</sup> the prostate cancer cells showed somewhat increased affinity toward marrow and competed with hematopoietic stem cells (HSCs) to engage the endosteal niche indicating that tumor used the HSC niche as metastatic niche. It was also shown that this tumor cell dissemination had already started early during tumor development and progression, even without any clinical signs of metastasis.<sup>[3]</sup> So, theoretically the BM involvement may be an unavoidable event in prostate cancer biology. Several studies carried out in variable number of prostate carcinoma and other solid tumor patients collectively to

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find out presence of micrometastases in BM by means of aspiration alone or by biopsy and aspiration both. All of them showed presence of cancer cells in significant number of prostate cancer patients' BM and recommended the use of BM trephine biopsy (over aspiration alone) with other ancillary techniques (like immunohistochemistry and bone scan) as practical and easy method to obtain prostate cancer cells, even if patient not have any related complaints.<sup>[6-9]</sup> Detection of disseminated tumor cells in BM of prostate cancer patients was also carried out by number of techniques including immunohistochemistry (IHC) staining with a pan-cytokeratin antibody<sup>[18]</sup> and by molecular techniques involving reverse-transcriptase polymerase chain reaction of BM samples from patients.<sup>[19]</sup> Pan cytokeratin is one of the marker which is not exclusive for prostate cancer and mainly used to ascertain presence of epithelial malignancy. On the contrary, reverse transcriptase although provides higher specificity but requires much more technical expertise and setup to carry out.

It was also stated that detection of micrometastases in BM aspirate at the time of primary diagnosis is an independent prognostic factor and it was suggested that it could have further potential applications like their use in monitoring therapeutic response or even in revealing targets for systemic therapies.<sup>[10]</sup> In case of prostate carcinoma this unique tumor material (trephine BM biopsy) had already been used and in future, could be utilized and reutilized to gain more in evolving knowledge of this particular disease.

In this study, we are trying to detect the metastatic cancer cells in BM of prostate biopsy proven and/or known metastatic prostate cancer patients with use of IHC markers, prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP), both of which are quite more specific for prostate. We are also trying to establish its correlation with biopsy Gleason score, 2010 AJCC clinical TNM 7<sup>th</sup> classification, and serum PSA level.

## AIMS AND OBJECTIVES

1. Detection of BM metastasis and micrometastasis in carcinoma prostate patient groups, using immunohistochemistry for PSA and PSAP
2. To correlate BM examination findings with prostate biopsy/TURP Gleason score, AJCC 2010, 7<sup>th</sup> TNM classification, and serum PSA levels.

## MATERIALS AND METHODS

The study was carried out at our department, in collaboration with department of urology of our institute for duration of 1 year from August 2011 to July 2012. All

histopathologically proven new cases and all known cases of metastatic/refractory/recurrent carcinoma prostate cases were included in the study by taking prior written consent on approved proforma and informing them about all complications related to procedure. All the cases that were not willing for BM examination were excluded. We performed BM examination from right superior iliac spine of 11 prostate cancer patients under all aseptic conditions. These 11 patients included both, cases without any known metastases, including seven patients and cases with known metastases to any site other than BM, including four patients.

Complete workup of all these patients with their hematological and biochemical parameter including hemoglobin, total leukocyte count, platelet count, and serum PSA estimation was carried out. We also inquired about their previous investigational records like computed tomography (CT)/MRI/bone scan and histo/cytopathological examination reports.

The BM aspiration films and trephine biopsy imprint films were stained with Leishman stain and were examined for metastatic tumor cells morphologically. BM clot specimens were fixed with fixative solution containing formaldehyde as main component. The trephine biopsy cores were put for overnight fixation followed by decalcification for 48 h as per the standard operating procedure carried out in our lab. The decalcifying solution which we used contained formic acid as decalcifying agent.

After appropriate decalcification, the biopsy specimen was put for overnight tissue processing along with clot section specimens. The sections of both trephine biopsy cores along with clot sections were stained with hematoxylin and eosin stain for morphological examination to look for metastatic tumor cells by three separate observers and results were noted.

Immunohistochemistry for PSA and PSAP were done on deparaffinized and rehydrated sections of trephine biopsy cores and clot sections. After antigen retrieval by heat method in appropriate buffer as per standard protocol, IHC staining for each marker was carried out. Positive and negative controls were also run with each batch simultaneously. The IHC sections were screened by three separate observers and results were noted. The statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 15.0 statistical analysis software.

## OBSERVATIONS AND RESULTS

Out of 11 patients of prostatic carcinoma, two (11.2%) showed metastases of tumor cells in bone marrow.

However, both of these patients were already had metastasis to distant site, but one of them had BM metastasis without any evidence of bone metastasis.

Patients having metastasis in BM showed very high serum PSA levels (>100 ng/mL) and prostate biopsy Gleason score  $\geq 7$ .

The metastatic tumor cells were identifiable morphologically on H and E sections and these were later confirmed immunohistochemically by application of markers PSA and PSAP [Figures 1-3]. The trephine biopsy, clot sections, BM aspiration, and imprint films did not show any specific efficiency and diagnostic utility over each other to detect such metastasis and all were positive for presence of metastatic tumor cells in each patient. The trephine biopsy sections provide an advantage of application of IHC technique. Both the immunohistochemical markers PSA and PSAP were equally sensitive and specific for detection of metastatic cancer cells in bone marrow. The important findings of the individual cases were presented in Table 1.

Some of the other findings which we found in our study are following:

- Patients having metastasis in BM have anemia, as common manifestation
- Aspiration from one of the two BM of metastatic cancer patient showed increased osteoblasts in the films.

## DISCUSSION

Bone metastases, characteristically osteoblastic, are the second most frequent site of prostatic carcinoma dissemination and at autopsy, pelvic bones, vertebrae, and ribs are the most common bones to harbor metastatic deposits.<sup>[1]</sup> Sometimes, this could be the only presenting feature of carcinoma prostate patient, if the tumor focus is small and located peripherally but high grade on histology. As compared to bone metastases, BM involvement is not very common finding in carcinoma prostate; but its

involvement is a sign of diffuse hematogenous spread of the malignancy as BM also does not have its lymphatic drainage. The resulting hematological manifestations of BM spread of tumor like anemia, leucopenia, or thrombocytopenia can add up to yield an overall worse prognosis for the patient.<sup>[6]</sup>

The bone involvement can be diagnosed by imaging modalities like CT, MRI, or bone scan; but definitive diagnosis of BM metastases depends on morphological or histopathological examination of BM. The prognostic significance of such BM involvement becomes more important in order to know the status of chemotherapeutic drug response (as majority of them have some amount of hematotoxicity) and also in patients who were once treated but after sometime presents with relapse of the disease in one or another form. Some independent researchers studied the detection and clinical relevance of BM micrometastases in epithelial malignancies and concluded that their significance in prognosis of various malignancies needs to be studied, especially for their early detection.<sup>[5]</sup> The newer AJCC TNM (7<sup>th</sup> edition) classification of breast cancer has incorporated this BM involvement in newer "M" subcategory but for the rest of malignancy, especially prostate cancer, this remains to be established.

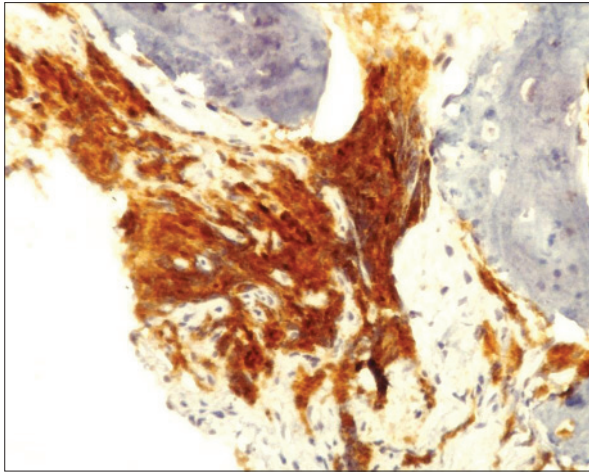
We had found presence of BM metastases morphologically and confirmed it by immunohistochemistry with PSA and PSAP, in 2 out of all 11 patients. Of all the four patients, who already had metastases, three of them had metastatic foci in bone on MRI and bone scan, while one had distant lymph node metastasis diagnosed on fine needle aspiration cytology, (there was no evidence of bone mets in this patient on any imaging investigation and patient also did not have any related complaints like bone pain, etc.). Interestingly, the BM metastases were found in patients, one with bone metastasis and other with lymph node metastasis (or without bone metastasis). On the contrary, one of the patient's BM, which harbors metastatic cells, revealed increased number of osteoblasts in the aspirate

**Table 1: Findings of individual cases enrolled in study**

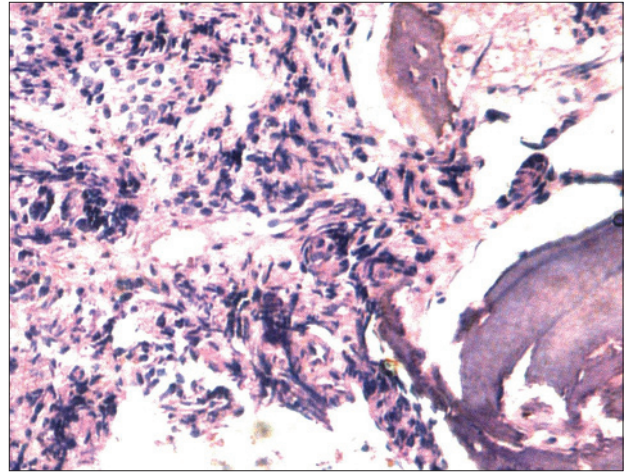
Pt serial no.	Age (in years)	TNM stage	Gleason score	Serum PSA (enrollment status)	Hb (g/dL)	Platelet count (cumm)	BM aspirate	Biopsy imprint	Clot section	BM biopsy	IHC-PSA BM	IHC-PSA clot	IHC-PSAP biopsy	IHC-PSAP clot
1	80	T2NXMX	6	61.9	10.5	182000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
2	60	T2cNXMX	8	>100	9	180000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
3	66	T3aNXMX	6	11.25	9	166000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
4	70	T2cNXM1b	7	234.4	10.5	170000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
5	60	T2cNXMX	6	0.36	8.5	160000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6	65	T2cNXMX	7	35.39	8	140000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7	63	T2cNXMX	7	12.45	8.5	168000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
8	58	T2cNXM1b	5	76.92	11.5	200000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
9	65	T2cN1M1a	7	149	8	182000	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive
10	65	T2cNXMX	6	17.83	11.5	200000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
11	59	T2cNXM1b	234	8.5	88000	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

BM: Bone marrow, IHC: Immunohistochemistry, PSA: Prostate-specific antigen, Pts: Patients, TNM: Tumor, node and metastasis, PSAP: Prostate-specific acid phosphatase

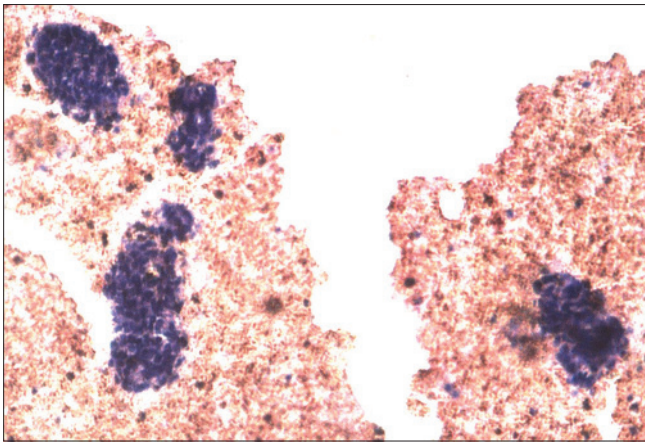




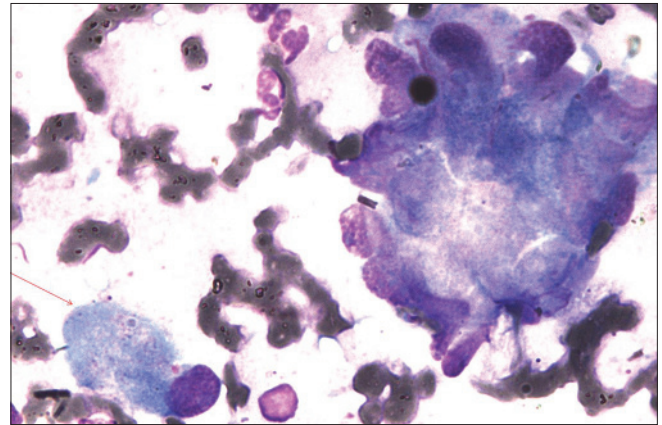
**Figure 1:** Immunohistochemistry section for prostate-specific antigen of same patient's trephine bone marrow biopsy section, showing positivity in tumor cells with adjacent bone trabeculae ( $\times 10$ )



**Figure 2:** Same patient's trephine bone marrow biopsy section, showing infiltration by tumor cells in marrow with adjacent bone trabeculae (H and E,  $\times 10$ )



**Figure 3:** Bone marrow clot section of patient, showing metastatic tumor cell clusters ( $\times 10$ )



**Figure 4:** Bone marrow aspirate film of patient showing metastatic tumor cell cluster, arrow indicates osteoblast (Leishman,  $\times 40$ )

film [Figure 4]. These findings indicate that BM metastases itself does not depend on presence of bone metastasis. Possibly, this BM dissemination may arise from different pathophysiological mechanism. Certain studies were carried out to know more about the biology of bone and BM metastases in prostate cancer patients. One of them stated that prostate cancer may use the stromal cell-derived factor-1 (SDF-1 or CXCL12; expressed by osteoblasts and endothelial cells) and its receptor (CXCR4) pathway to spread to bone.<sup>[12]</sup> In another study researchers tried to examine, whether metastatic prostate cancer cells can alter regulation of normal bone and marrow development by HSCs and hematopoietic progenitor cells (HPCs) and they found that HSC/HPCs can act as novel targets for future therapy involved in the bone abnormalities of prostate cancer as different types of osteoblastic and osteoclastic responses were noted in the animals when they were inoculated with different cell lines isolated from nonmetastatic and metastatic tumor cells.<sup>[13]</sup> Logothetis

and Lin<sup>[16]</sup> also stated that interaction between host cells and metastatic prostate cancer cells, which tend to be osteoblastic rather than osteoclastic, was an important component of organ-specific cancer progression and this knowledge can lead to development of more effective therapies in future.

As we had small sample size with only two positive cases in our study of 1 year duration, the efficacy and diagnostic utility of procedures including BM aspiration, clot sections, trephine biopsy, and imprint films did not show significant association to detect metastases in BM, over each other. However, the trephine biopsy and clot section specimens are more useful in comparison to aspiration alone for detection of metastasis and micrometastasis because of application of immunohistochemical markers, like PSA and PSAP in our cases. These biopsy and clot section paraffin blocks can also be retrieved in future in order to know the status of any other immunohistochemical marker or to carry out tissue microarray study. Brunning *et al.*,<sup>[14]</sup> in a retrospective study involving 101 patients

of various neoplastic diseases, stated that BM aspiration alone, most of times, was not able to detect the focal tumor cells aggregate in many malignancies like lymphoma and metastatic epithelial cancers. In a 5-year study, Sharma and Murari<sup>[15]</sup> had detected metastasis in 25 BM examination and demonstrated the usefulness of combining trephine biopsy with aspirate examination for increased detection of BM metastasis. Thus, biopsy and clot section with aspiration, right now, provide one of the most effective way to detect metastatic cancer cells in BM.

Both the patients, who had BM metastasis, had serum PSA levels >100 ng/mL at the time of BM examination and had Gleason score 7 on TRUS-guided prostate biopsy histopathological examination. Both of these findings were consistent with the established facts that very high serum PSA levels and higher Gleason score are more likely to be associated with metastases of carcinoma prostate.

Among the other parameters, both of these patients show hematological alterations in the form of anemia (mean hemoglobin was 8.0 g/dL) at the time of BM examination. This can be one of the problematic factors for the clinician in order to provide effective chemotherapeutic treatment to the patient and have adequate and sustained response. As far as TNM staging was concerned, the patients who had BM metastases were of stage T3a and T2c. As per the guidelines,<sup>[17]</sup> the results were consistent and showed that BM dissemination of cancer cells was more likely in high-risk prostate cancer patients. In case of both immunohistochemical markers, PSA and PSAP, which we used in our study, both were able to detect metastatic cancer cells in BM clot sections as well as trephine biopsy sections with equal efficacy.

Thus, BM dissemination of metastatic prostate carcinoma cells can be present in advanced carcinoma patients. This finding may be associated with other hematological discrepancies, as in our case it was anemia, but for prognostic significance of such findings and effect on median survival of such patients, a large sized study with long-term follow-up of the patients is required. Aspiration along with clot sections and biopsy can provide an easier, cheaper, and more specific modality to detect BM metastases and micrometastases and follow-up these patients. One of the significant and important risk associated with this modality is the invasive nature of this investigation which can sometimes create anxiety and may compel the patient to withdraw. However, proper counseling and conversation with patient and relatives can not only overcome these hurdles but can also add much more satisfaction in the mind of patient along with giving information about his disease progression.

We conclude that presence of metastatic cancer cells in BM is not only providing evidence of diffuse hematogenous spread of prostate cancer but it also opens new horizons that can lead to study of expression of different stromal factors, proteins, cancer-related genes, and behavior of osteoblasts and related cells in this easily available source material to understand more about prostate cancer progression. Above all, this can be of paramount importance in patients regarding their disease prognosis and long-term disease free survival, if they are followed-up for long duration and may include, in future, development of more effective therapies.

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