

Immunohistochemistry: Not as Sensitive as Expected for Detecting Bone Marrow Micrometastases in Cases of Epithelial Malignancies?

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ABSTRACT

Background: Bone marrow is the most common site of metastases of epithelial malignancies from breast, lung, colon, prostate, ovaries and endometrium. These have poor prognosis as early tumor cells dissemination to bone marrow leads to poor treatment response. Bone marrow involvement by tumor cells can be seen on microscopic bone marrow examination and confirmed by immunohistochemistry. In this prospective observational study, we plan to analyze the incidence of bone marrow dissemination in epithelial malignancies and estimate the utility of immunohistochemistry in detecting micro metastases and correlate it with other clinicopathological parameters.

Methods: clinical details and complete hematological workup was done in 31 new cases of epithelial malignancies during our study period of one year. Bone marrow aspiration, bone marrow biopsy and clot section were done and analyzed morphologically and immunohistochemically using CK 7 and CK 20.

Result: out of a total of 31 cases of epithelial malignancies, 2 cases of prostate carcinoma showed positive metastatic cell cluster and 2 cases of breast carcinoma and 1 case of lung carcinoma showed dispersed atypical cells in bone marrow.

Conclusion: Immunohistochemistry did not show any observed benefit than routine microscopy in diagnosing the bone marrow metastases. Small sample size and lack of ancillary techniques like polymerase chain reaction (PCR) are drawback in the diagnosis of isolated tumor cells, micro metastases and confirmation of the results obtained on immunohistochemistry. Extensive studies are required to elucidate the pathogenetic pathway and clinical implications of bone marrow metastatic cells for the diagnosis, staging and treatment of epithelial malignancies.

Keywords: Bone Marrow Metastases, Immunohistochemistry, Bone Marrow Examination, Epithelial Malignancies.

Introduction

The most common malignancies are derived from simple epithelia of breast, lung, colon, prostate, ovaries, and endometrium. Carcinomas from these sites usually metastasize to regional lymph nodes and other organs, such as lung, brain, liver and bones. Among various epithelial malignancies breast (28.8%) and lung (23.3%) are common to metastasize to bone marrow [1,2]. The diagnosis of the metastatic carcinoma can be very difficult. The determination of the primary site of the metastasis is a challenge to both oncologists and pathologists, having potential important clinical and therapeutic consequences [3]. Bone metastasis is a catastrophic complication for intractable pain and other clinical problems such as fracture after trivial injury, spinal cord compression, and hypercalcemia; it also signifies that the malignant process is incurable [4]. More than 30 years ago Sloane et al (1980) [4] introduced the term 'micro metastasis' to describe isolated disseminated tumor cells which are not detected by conventional staging methods. Micro metastases are microscopic deposits (less than 2mm) of

malignant cells that are segregated spatially from the primary tumor and depend on neo vascular formation (angiogenesis) to propagate. It was concluded by some researchers that the concept of micro metastasis had resulted in paradigm shift in the staging of epithelial tumours and our overall understanding of malignant processes. Imaging techniques like radionuclide bone scan, magnetic resonance and computed tomography are usually ineffective in detecting bone marrow metastases and bone marrow aspiration and biopsy remains the procedure of choice to document bone marrow involvement; It is the fastest, easiest and cheapest method to search for metastatic foci in the bone marrow [5,6]. With regard to detection techniques, most investigators have used immunocytochemical assays with a variety of 'epithelial specific' cytoskeleton and membrane antigens. CK 7/20 have been found to be positive in most of the epithelial malignancy and hence shows great propensity for differentiation of various epithelial malignancies [7,8]. In this study, we are trying to assess the prevalence of bone marrow metastases of epithelial malignancies coming to

our department over a period of 1 year and also to detect the sensitivity of immunohistochemistry in detecting these micrometastatic foci in bone marrow biopsies. We are also trying to study the various clinicopathological parameters and to decipher any significant correlation between them and bone metastases.

Materials and Methods

In this prospective observational study of one year duration, 31 biopsies proven new cases of epithelial malignancy reporting to the department of surgical oncology were included after taking prior written consent on approved proforma by the ethical committee and informing them about all complications related to procedure. Patients on chemotherapy, Patients with coagulation disorder or patients not consenting for the study were excluded. Identified cases of epithelial malignancies were asked personal identification details, disease symptoms, any presenting signs and symptoms related to cytopenias like fatigue, dizziness, orthostatic hypotension, bleeding, infections and data about the investigations already done. The bone marrow examination was carried out in the departmental laboratory after taking written consent from all the subjects under all required precautionary measures, from the posterior superior iliac spine(right). The examination included trephine biopsy and BM aspiration along with preparation of clot sections and touch imprint of the same patient. Bone marrow aspiration smears and imprints were stained with Leishman stain and examined for metastatic cells. The rest of the aspirate was put to clot and further processed with formalin (10%) for clot sections [17,18]. Bone biopsy was decalcified as per the protocol of our department and then processed along with the clot section. Both the sections were stained with hematoxylin and eosin stain and examined for metastatic tumor cells. EDTA sample was collected for haemogram and sample in plain vial was collected for serum lactate dehydrogenase (LDH) and serum alkaline phosphatase (ALP) estimation. Paraffin sections were deparaffinised and rehydrated. After antigen retrieval by microwave method in ready to use antigen retrieval solution as per the standard protocol; IHC staining for CK7 and CK20 were done [19,20]. Control samples were also run along with the IHC and sections were screened and reported by three separate observers. The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software.

Result

Primary Site of Involvement: Of all the 31 cases, 13 cases (41.94%) were of breast carcinoma, 7 cases

(22.58%) were of gall bladder carcinoma, 4 cases (12.90%) each were of lung and gastro intestinal malignancy and 3 cases (9.68%) were of prostate carcinoma (graph 1).

Complain of Bone Pain: out of 31 cases only 5 cases (16.13%) gave history of bone pain (graph 2).

Radiological Evidence of Bone Metastases was present in 4 cases (12.90%) out of which only 2 cases showed tumor cell clusters on bone marrow examination.

Cytopenias: Serum hemoglobin levels of the study subjects ranged from 5.20-13.60 gm/dl. TLC levels ranged from 3600-12500/cumm with a mean value of 7216+2545/cumm. Median TLC level was found to be 6800/cumm. Platelet count levels ranged from 40000-380000/cumm with a mean value of 219774+75747/cumm. Median PTLC level was found to be 220000/cumm.

Serum LDH and ALP showed variable result and no concordance was observed between the positive cases with bone metastases and the remaining negative cases.

Bone Marrow Examination: 2 cases showed bone marrow metastatic cell cluster in BMA, BMB and clot section. Three cases showed isolated suspicious cells on bone marrow aspirate.

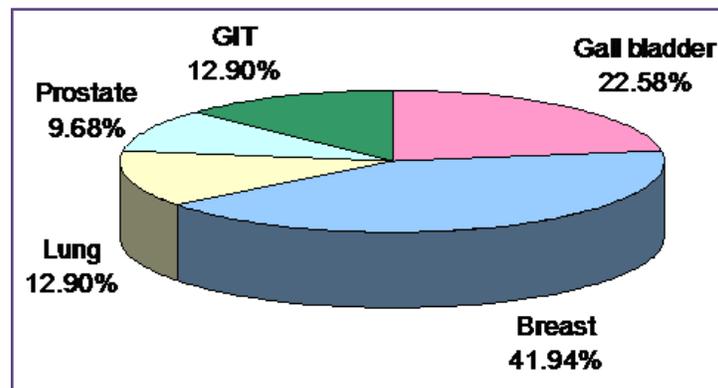
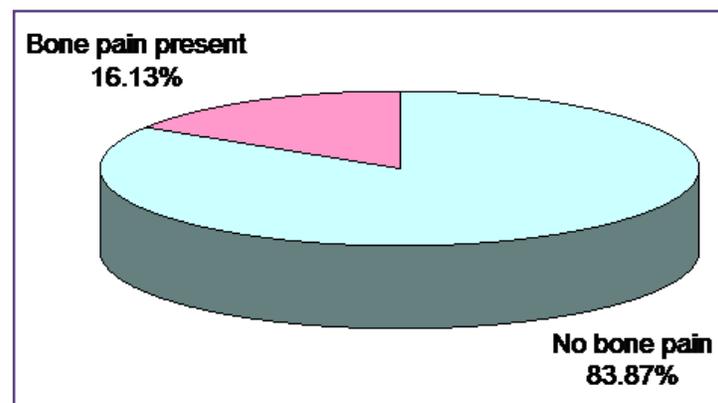
Immunohistochemistry: cases with metastatic cell cluster showed negative CK7 and CK 20 but positive PAS and PASP stain on IHC. The cases with isolated suspicious metastatic cells showed inconclusive result with CK 7 and CK 20 (table 2).

Discussion

Bone is the third most common site of metastasis after lung and liver. Breast and prostate carcinomas frequently metastasize to bone. Sometimes this could be the only presenting feature of epithelial malignancy (this feature is more observed in prostatic carcinoma and carcinoma lungs). In such cases the tumor focus is small and located peripherally but on the histopathological evaluation the lesion is of high grade. As compared to bone metastasis, bone marrow involvement is not very common finding in any epithelial malignancy; but its involvement is a sign of diffuse hematogenous spread of the malignancy. The resulting hematological manifestation of bone marrow spread of tumor like anemia, leukopenia or thrombocytopenia can add up to yield an overall worse prognosis for the patient [9]. Tumor cells commonly metastasize to the most vascularized parts of the skeleton, particularly the red bone marrow of the axial skeleton and the ends of the long bones, the ribs, and the vertebral column

Table 1: Age wise distribution of study population

Age Group	Number of subjects	Percentage
25-35	4	12.90
36-45	7	22.58
46-55	11	35.48
56-65	9	29.03
Total	31	100
Mean±SD (Range)	49.65±10.48 (25-65)	

Graph 1: Primary Site wise distribution of study population**Graph 2: Bone pain in Study Population****Table 2: Metastatic Positivity Status for different diagnostic tests used (n=31)**

SN	Name of test	No. positive	% Positivity
1.	BMA	3*	9.68
2.	Biopsy imprint	2	6.45
3.	Clot section	3*	9.68
4.	Bone biopsy	2	6.45
5.	CK-7 B Biopsy	0	0
6.	CK-7 Clot Section	0	0
7.	CK-20 B Biopsy	0	0
8.	CK-20 Clot Section	0	0

*includes suspicious (3 each)

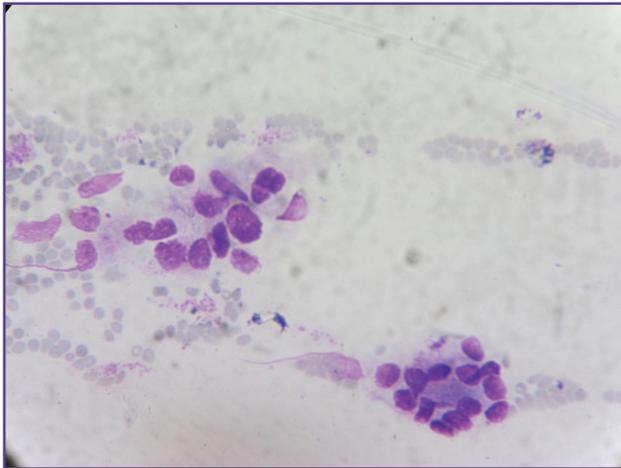


Fig 1: Tumor cell cluster on bone marrow aspirate; 40X, Leishman stain.

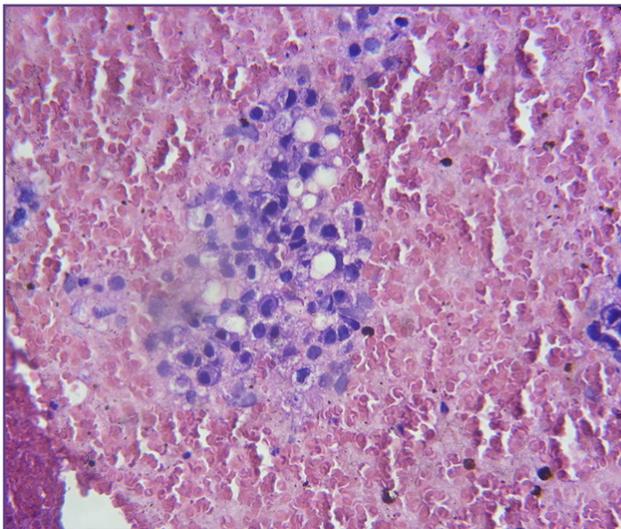


Fig 2: Tumor cell cluster on clot section; 10X, H&E stain.

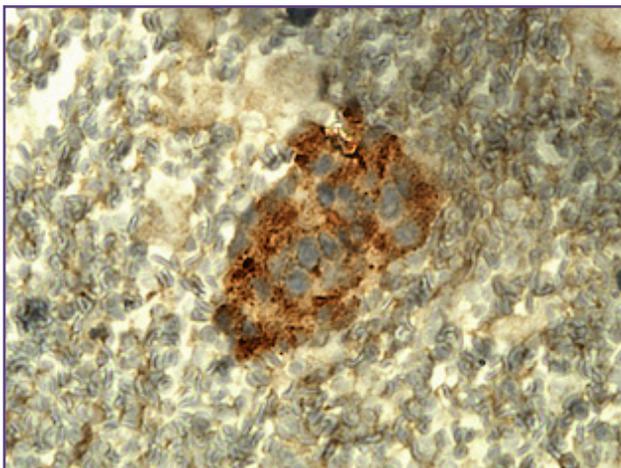


Fig 3: Tumor cell cluster on IHC PSA positive;40X

^[10]. Once tumor cells become housed in the skeleton, cure is no longer possible and only palliative therapy is available. Acidosis is the increased acidity in a given location, whether it is blood, urine or tissues. Osteoclasts generate extracellular protons, lowering the pH of the extracellular matrix (ECM) around the Osteoclasts to approximately 4.5^[10]. Nociceptors in the bone trigger a pain response in the brain in response to this acidosis. It is thought that this is the primary source of the dull, chronic pain experienced by patients with bone metastasis. The uncoupled regulation of the Osteoclasts and osteoblasts leads to malformation of the bone. Malformed bones are unable to withstand the normal mechanical stresses placed on them in day-to-day activity, leading to fractures, spinal compression, and spinal instability. Malformed bones may also mechanically trigger pain receptors both within the bone and in the surrounding tissue. The bone involvement can be diagnosed by imaging modality like CT, MRI or bone scan but definitive diagnosis of bone marrow metastasis depends on morphological or histopathological examination of bone marrow.

The prognostic significance of such bone marrow involvement becomes very important in order to know the status of chemotherapeutic drug response as most of these drugs are hematotoxic and also to evaluate the patients who were once treated and were in remission stage but after some time presents with relapse of the disease in one or the other form. Further studies have enhanced our knowledge with the inclusion of terms like isolated tumor cells and micro metastasis^[11]. The term “occult” metastasis has been defined as one missed by initial histological examination and identified on subsequent assessment, and also as a metastasis identified through additional evaluation of paraffin embedded lymph node blocks^[11]. Isolated tumor cells (ITCs) are not greater than 0.2 mm and are associated with qualitative features as follows: they have no malignant activity (e.g. no proliferation, no stromal reaction) and are located in lymphatic sinuses. ITCs should be named micro metastasis when located outside the lymphatic sinuses, i.e., in the nodal tissue. The identifier (i) is used to indicate ITCs that are usually detected by immunohistochemistry but also may be verified with hematoxylin and eosin (H&E) stain. Micro metastasis is defined as having a size of more than 0.2 mm but not greater than 2 mm.

The newer AJCC TNM (7th edition) classification of breast cancer has incorporated this bone marrow involvement in newer “M” subcategory but for the rest of the malignancies, this remains to be confirmed^[11,12]. In our study, we tried to detect metastasis and micro metastasis status in bone marrow of biopsy proven cases of epithelial

malignancies patient, using immunohistochemistry for CK7 and CK 20.

We found presence of bone marrow metastasis morphologically as well as confirmed by immunohistochemistry in 2 out of 31 cases. Total of 3 prostate malignancy patients were taken into the study group. The 2 positive cases belonged to the prostate malignancy as was confirmed on further workup using immunohistochemistry PSA and PSAP for confirmation. These cases had history of bone pain suggestive of bone involvement with one patient having evidence of distant lymph node metastasis proven on fine needle aspiration cytology. One patient of prostate malignancy did not have history suggestive of bone marrow involvement. One patient with breast carcinoma gave symptomatic history suggestive of bone metastasis along with bone scan suggestive of bone metastasis. In this patient, the bone marrow biopsy showed some suspicious clusters which could not be neglected but the immunohistochemistry on the bone biopsy of this patient came out to be inconclusive. The bone marrow aspiration from this patient showed reactive changes in the form of increased plasma cell and eosinophils. One patient with lung carcinoma had history of bone pain along with radiological evidence of bone marrow metastasis but the bone marrow examination from right posterior iliac spine came out negative. This could imply that bone marrow metastasis itself does not depend on presence of bone metastases or bone metastases does not guarantee the metastasis to bone marrow. Possibly, this bone marrow dissemination may arise from different pathophysiological mechanism.

As far as the efficacy and diagnostic utility of procedures including bone marrow aspiration, clot sections, trephine biopsies and imprint films were concerned, there was no significant association found to detect metastasis in bone marrow, over each other, as we had small sample size with only two positive cases in our study of one year duration. Earlier the studies carried out for the detection of bone marrow metastasis were done on large scale and spread over a long duration of 3-5 years follow up basis or retrospectively, investigating past institutional records. Most of the studies with significant results were done on multi-institutional collaboration basis [4,9,13,14,15]. In our study among the different epithelial malignancies taken into consideration majority, 13 cases were of breast malignancy (41.94%), 7 were of gall bladder malignancy, 4 each of lung and gastrointestinal tract malignancy and least cases, 3 were of prostate malignancy (9.68%). This is in accordance with the result of Mohanty et al [13] who detected that among all the epithelial malignancy they studied; prostate cancer had the maximum incidence of

bone marrow metastasis. This finding also corroborated with Manish et al [16] who conducted a similar study in prostate malignancy at this institute. In our study, most of the malignancies were present in the older age group, 46-55 years (35.48%) (Table 1). This is in concordance with the data provided in the Harrison's principles of internal medicine (18th edition) [1]. Most of the cases were of malignancies in female patients (74.19%). This is due to the fact that most common malignancy for which a patient reports to our health facility is carcinoma breast. Carcinoma gall bladder is also relatively more common in female patients. This study also suggested that bone pain in itself is a subjective assessment and cannot be relied upon to predict bone metastasis of the epithelial malignancy. There was no significant association present between leucopenia or thrombocytopenia in these patients. Serum Lactate dehydrogenase levels assessed in our cases showed a mean value of 399 IU/L, this was raised in many malignancies but was also normal in certain cases. Therefore, it may be suggested that the levels of serum LDH correlates with the stage of the malignancy. Elevated S. ALP levels were seen in most of the patients. Raised value can give an insight for the thought of bone metastasis but it was not substantiated in our study.

As was mentioned by Saddettin Kilickap et al. 2007[7], in a study involving total 73 cases of solid tumors, out of which 5 were prostate cancer, biopsy as compared to aspiration alone, with immunohistochemistry or molecular techniques may be needed and more useful in detection of microscopic tumor burden in patients of any epithelial malignancies.

As for the immunohistochemistry used CK 7 and CK 20 were used to detect the unknown primary. These were unable to detect the presence of prostate metastasis as both these markers are negative in prostatic carcinoma and we had to rely on PSA and PSAP for confirmation. More so over, two cases of breast carcinoma and one case of lung carcinoma showing suspicious cells on bone marrow aspiration and clot sections remained inconclusive with immunohistochemistry also; clearly indicating the inefficacy of immunohistochemistry in providing any diagnostic benefit over routine microscopy.

BM dissemination of metastatic epithelial malignancy can be present in advanced carcinoma patients. This finding may be associated with other hematological discrepancies like anemia, elevated S. ALP, or presence of increased osteoblasts in bone marrow biopsies, but for their significance, prognostication and effect on median age of survival of such patients, a large sized study with

long term follow up of patients is required. Aspiration along with biopsy can provide an easier, cheaper and more specific and effective method to detect and follow up of such patients.

Conclusion

We conclude that presence of cancer cells in bone marrow not only indicates a seed disseminated from the advanced stage spreading malignancy but this seed will give as an idea to perform more stringent and in-depth researches to look for new parameters, indicator, proteins involved in the genesis and mechanism of the cancer cell progression and spread. Also, immunohistochemistry does not provide any diagnostic benefit over routine microscopy in diagnosing bone marrow micro metastases and further ancillary techniques are required for the detection of micro metastases and isolated tumor cells. This may have a significant and beneficial role on the survival of cancer patients.

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