RESEARCH COMMUNICATION

Occult Micrometastasis to Bone Marrow in Early Lung Cancer: A Clinicopathologic Study from West Bengal, India

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Abstract

Although bone marrow micrometastasis may remain silent, its detection changes the staging and management of lung cancer. In the present study conducted in West Bengal, India, 74 diagnosed bronchogenic carcinoma cases (28 squamous cell carcinomas, 20 adenocarcinomas, 9 small cell carcinomas, 4 large cell carcinomas, 13 unclassified) in early stages (stage I, II and IIIA) were included. Complete hemograms, bone marrow aspiration and cell blocks of aspirated material, trephine biopsy were done for detection of micrometastasis. Overall micrometastases in bone marrow were noted in 17 cases (23.0%). We detected marrow metastasis in 44.4% cases of small cell carcinomas and 21.2% cases of non small cell lung cancer (50% of large cell carcinomas, 20% of adenocarcinomas, 17.9% of squamous cell carcinomas) and 15.4% cases of unclassified carcinoma. We found a statistically significant correlation between marrow metastasis and low platelet count (P=0.0001) and high ESR (P=0.0003), but no significant correlation with hemoglobin percentage (P=0.36), total leukocyte count (P=0.58) and eosinophil count (P=0.44). A definite correlation noted between micrometastasis with the clinical stage (no case in Stage I, 12.5% in Stage II, 30.4% in Stage IIIA patients). We emphasize that detection of micrometastasis is essential particularly in non small cell cancers, where treatment with curative intent is planned, which can be suitably done by morphological study of bone marrow aspirate and biopsy in countries like India.

Keywords: Lung carcinoma - micrometstasis - bone marrow aspiration/ biopsy - hematological parameters - clinical stage

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Introduction

Bone marrow is one of the most common organs involved by tumors that metastasize by blood stream (Sharma et al., 2003). It is imperative to rule out bone marrow involvement in malignancies where treatment with curative intention is being considered. Bone marrow infiltration may be suspected on the basis of bone pain, pathological fracture, lytic or sclerotic lesions on x-ray, hypercalcemia, elevated serum alkaline phosphatase or unexplained hematological abnormalities (Sharma et al., 2003). However, metastasis may be present in bone marrow without any abnormalities being recognized in bone scans, radiographic picture, serum chemistry or hematological parameters (Hansen et al., 1971). Thus, despite the development of technologies allowing for a more accurate staging like computerized tomography (CT) scan, magnetic resonance imaging (MRI), nuclear/ radionuclide scanning, and various preoperative surgical diagnostic procedures, no major breakthrough has been made in improving long-term survival in lung cancer (Poncelet et al., 2001). Such low survival rate is due to several reasons like, the existing histologic technique of pathological examination of node for the detection of metastasis is insufficiently sensitive and the mediastinal lymph node sampling during surgery is inadequate. Early dissemination of metastatic cells may remain undetected during the disease evaluation (Poncelet et al., 2001). Since the frequency of metastatic spread varies among the four major histological types of carcinoma, analysis of data by histological type might be helpful. (Hansen et al., 1971). The frequent metastasis to the bone marrow has contributed to the concept that small cell anaplastic carcinoma is a disseminating disease which the majority of the patients have at the time of diagnosis (Hirsch et al., 1980). The presence of two or more micrometastatic cells in the bone marrow, even in absence of negative nodes (PN0) is a strong predictor of recurrence, and has a statistically significant impact on disease-free and overall survival (Poncelet et al., 2001).

We have performed bone marrow aspirates and biopsies and evaluated hematological findings of patients with early stages (stage I, II, IIIA) of bronchogenic carcinomas where treatment with a curative intention was considered. Aims of the present study were to investigate the prevalence of bone marrow micrometastasis according to the type and stage of disease. Correlation of different stages with the haematological profile was also evaluated.

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Materials and Methods

The present study was performed in NRS Medical College, Kolkata, West Bengal, India over a duration of 2 years. All newly diagnosed cases of histologically and /or cytologically documented bronchogenic carcinoma were evaluated. Patients with clinically stage IIIB and IV disease were excluded as the distal spread from the primary tumor has already been identified. Applying these criteria, 74 patients with bronchogenic carcinoma were enrolled in the study. Investigations required for the study were done before beginning of specific therapy. A written informed consent was obtained for invasive procedures.

The diagnosis of lung carcinoma was established by histologic examination of bronchoscopic biopsy specimens, CT guided FNAC of lung mass and sputum cytology. Staging of lung cancer was done by comprehensive clinical examination, radiological evaluation included chest X-ray PA view, CT scan of thorax and USG of abdomen, bronchoscopy, biochemical tests and other investigations as demanded by the cases.

All patients underwent complete hemogram, and bone marrow aspiration and trephine biopsy from posterior superior iliac spine and/or iliac crest. Smears of bone marrow aspirate were stained with Leishman stain. Cell blocks were prepared from marrow particles obtained by aspiration and histological sections were prepared. Formalin fixed biopsy specimen was stained with Hematoxylin and Eosin (H&E). The diagnosis of bone marrow metastasis was based upon finding of the malignant cell in either in the smears of aspirate, cell block or biopsy or any one. Results were analyzed using standard statistical methods.

Results

The study group included 68 men and 6 women with female male ratio 1: 11.33. The age range varied from 30 to 76 years. The histological types of lung carcinoma were (Table 1) squamous cell carcinoma (28 cases, 37.84%), adenocarcinoma (20 cases, 27.03%), small cell carcinoma (9 cases, 12.16%) and large cell carcinoma (4 cases, 5.41%). 13 cases (17.57%) could not be classified on morphological basis and these cases were designated as unclassified.

The most common presenting feature was (Table 2) chest pain (56 cases, 75.68%) followed by cough (49 cases, 66.22%), hemoptysis (38 cases, 51.35%), breathlessness (27 cases, 36.49%) and fever (27 case, 36.49%). Most of the cases were either in clinical Stage IIIA (46 cases, 62.16%) or II (24 cases, 32.43%). Only four cases (5.41%) were in stage I.

In the study group, micrometastasis in bone marrow was noted in 17 cases (23.0%). Both bone marrow trephine biopsy and aspirate detected malignant cells in 14 cases (82.35%) while only trephine biopsy detected malignant cells in three cases (17.7%). Among the different histological types (Table 3), large cell carcinoma cases had 50% rate of micrometastasis followed by adenocarcinoma (20.0%) and squamous cell carcinoma (17.86%). As a group, non small cell carcinomas (squamous cell carcinoma, adenocarcinoma, large cell carcinoma) had 11 cases of marrow micrometastasis (21.15%). Small cell carcinoma had 44.44% and unclassified group had 15.38% micrometastasis. In majority of patients with marrow metastasis, bone marrow aspiration smears and cell blocks showed cohesive clusters of malignant cells (Figure 1) while trephine biopsy detected clusters as well as isolated malignant cells (Figure 2). Bone marrow showed hypercellularity in 21 cases (26.38%) and decreased cellularity in 5 cases (6.76%).

In 59 patients total leucocyte counts (TLC) were within normal range. Leucocytosis was seen in 14 patients. Leucopenia was seen in only one patient. Hemoglobin percentage (Hb%) was lower in cases with marrow

Table 1. Demographic Data According to the Histological Types of Lung Carcinoma

Type of carcinoma	Number of patients(%)	Mean age in year	Female male ratio
Squamous cell carcinoma	28 (37.8)	58.2	2: 26
Adenocarcinoma	20 (27.0)	56.8	2: 18
Small cell carcinoma	9 (12.2)	56.1	0: 9
Large cell carcinoma	4 (5.4)	60.5	0: 4
Unclassified	13 (17.6)	58.8	2: 11
Total	74	57.8	6: 68

Table 3. Bone Marrow Micrometastasis in Different Types of Bronchogenic Carcinoma

Type of carcinoma	Number of patients	Bone marrow micrometastasis	Percentage
Squamous cell Carcinoma	28	5	17.86
Adenocarcinoma	20	4	20.00
Small cell carcinoma	9	4	44.44
Large cell carcinoma	4	2	50.00
Unclassified	13	2	15.38
Total	74	17	22.97

Table 2. Symptoms Noted among the Cases of Different Types of Lung Carcinomas

Type of carcinoma	Clinical features (Percent)					
	Chest pain	Breathlessness	Cough	Fever	Hemoptysis	Weight loss
Squamous cell carcinoma	23 (82.14)	14 (50.00)	20 (71.43)	8 (28.57)	17 (60.71)	1 (3.57)
Adenocarcinoma	15 (75.00)	5 (25.00)	13 (65.00)	8 (40.00)	8 (40.00)	1 (5.00)
Small cell carcinoma	5 (55.56)	4 (44.44)	8 (88.89)	5 (55.56)	5 (55.56)	1 (11.11)
Large cell carcinoma	4 (100.00)	2 (50.00)	3 (75.00)	1 (25.00)	3 (75.00)	0 (0.00)
Unclassified	9 (69.23)	2 (15.38)	5 (38.46)	5 (38.46)	5 (38.46)	2 (15.38)
Total	56 (75.68)	27 (36.49)	49 (66.22)	27 (36.49)	38 (51.35)	5 (6.76)

Table 4. Comparison of Different Hematological Parameters in Patients with and without Bone Marrow Micrometastasis

Type of carcinoma	Mean (Standard deviation)					
	Hb (gm/dl)	Platelet ($x10^3/mm^3$)	Total leukocyte count (per mm^3)	Neutrophil	Eosinophil	Erythrocyte sedimentation Rate
Squamous cell carcinoma						
All cases	10.5(±1.7)	256.6(±20.3)	9938.5 (±3209)	71.6(±8.7)	3.8(±3.1)	69.2(±34.0)
Without marrow micrometastasis	10.6(±1.8)	268.1(±14.8)	9995.5(±3164.5)	71.0(±8.9)	3.9(±3.3)	60.8(±29.3)
With marrow micrometastasis	9.6(±1.0)	228.7(±18.5)	9625.0(±3944.9)	75.3(±7.0)	2.7(±2.1)	116.7(±11.5)
Adenocarcinoma						
All cases	9.9(±3.0)	246.7(±24.7)	8317.6(±1967.9)	64.9(±9.1)	6.7(5.0)	45.4(±29.4)
Without marrow micrometastasis	10.0(±2.6)	254.2(±20.3)	8484.6(±2164.4)	65.5(±9.7)	6.4(±5.0)	44.3(±30.9)
With marrow micrometastasis	9.5(±5.0)	236.6(±21.5)	7775.0(±1184.3)	62.3(±6.8)	8.0(±6.0)	55.0(±36.6)
Small cell carcinoma						
All cases	10.9(±2.4)	234.8(±18.6)	8062.5(±3469.8)	68.5(±7.4)	5.9(±3.0)	51.4(±49.5)
Without marrow micrometastasis	11.4(±1.7)	246.3(±10.7)	6620.0(±3625.9)	68.0(±6.8)	6.0(±2.8)	27.5(±12.6)
With marrow micrometastasis	10.2(±3.7)	212.4(±30.4)	10466.7(±1404.8)	69.3(±9.9)	5.7(±4.0)	83.3(±66.6)
Large cell carcinoma						
All cases	11.5(±1.0)	236.6(±28.3)	8650.0(±1841.2)	57.0(±13.9)	7.0(±1.4)	61.7(±48.6)
Without marrow crometastasis	11.6(±0.06)	242.8(±20.4)	8550.0(±70.7)	57.0(±1.4)	7.5(±0.7)	20.0((±14.6)
With marrow micrometastasis	11.3(±1.7)	222.7(±22.9)	8750.0(±3182.0)	57.0(±24.0)	6.5(±2.1)	82.5(±46.0)
Unclassified						
All cases	10.3(±3.6)	244.6(±24.8)	8346.2(±1618.4)	65.5(±8.7)	5.7(±3.0)	50.6(±37.8)
Without marrow micrometastasis	10.7(±4.2)	240.4(±20.1)	8400.0(±1736.5)	64.1(±9.3)	4.7(±2.7)	45.8(±41.8)
With marrow micrometastasis	9.3(2.4)	252.3(±40.6)	8166.7 (1443.4)	70.0(±5.3)	9.0(±1.0)	70.3 (±32.6)
Among all types						
All cases	10.4(±2.4)	244.4(±26.2)	8932.4(±2701.2)	67.5(±9.6)	5.3(±3.7)	58.6(±36.8)
Without marrow micrometastasis	10.5(±2.3)	260.9(±16.7)	8884.9(±2805.8)	67.4(±9.2)	5.1(±3.7)	50.4(±30.6)
With marrow micrometastasis	9.9(±2.7)	232.3(±36.4)	9300.0(±2429.8)	67.5(±10.9)	5.9(±4.0)	86.0(±43.7)

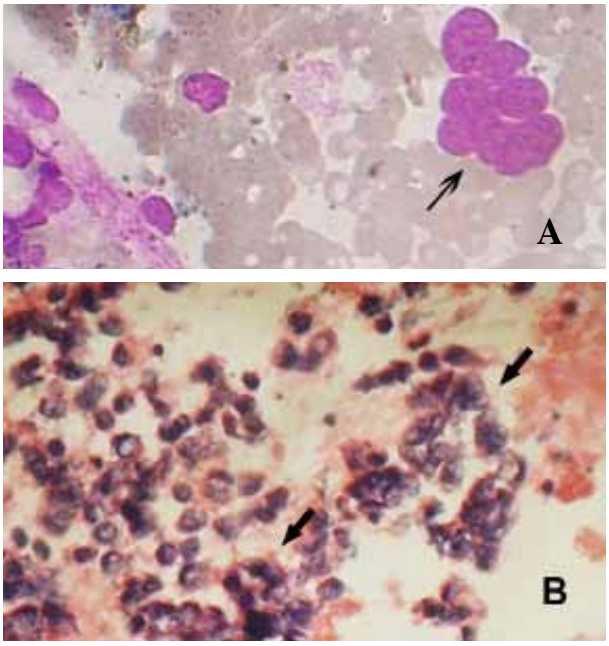

Figure 1. Clusters of Malignant Cells in a Bone Marrow Aspirate A. Smear (Leishman, x400) B. Cell Block (H&E, x400)

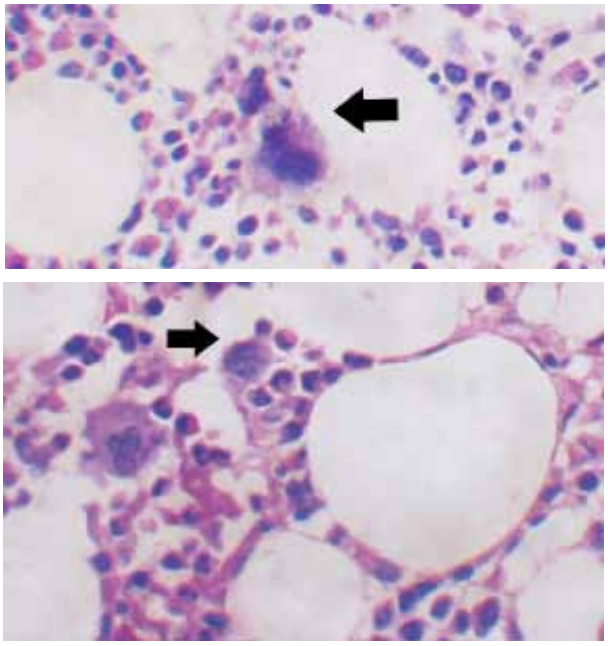

Figure 2. Scattered Malignant Cells in a Bone Marrow Biopsy (H&E, x400)

Table 5. Statistical Significance of Different Hematological Parameter and Bone Marrow Micrometastasis

Parameter	No marrow micrometastasis (n=57)			With marrow micrometastasis (n=17)			t value	Two-tailed P value	Interpretation of Unpaired t test results
	Mean	Standard deviation	Standard error of the mean	Mean	Standard deviation	Standard error of the mean			
Hemoglobin (gm/dl)	10.5	2.3	0.305	9.9	2.7	0.655	0.9067	0.3676	Not statistically significant
Platelet ($x10^3/mm^3$)	260.9	16.7	2.212	232.3	36.4	8.828	4.5767	0.0001	Extremely statistically significant
Total leukocyte count (per mm^3)	8884.9	2805.8	371.637	9300.0	2429.8	589.313	0.5509	0.5834	Not statistically significant
Eosinophil (percent)	5.1	3.7	0.490	5.9	4.0	0.970	0.7681	0.4449	Not statistically significant
ESR (mm per hour)	50.4	30.6	4.053	86.0	43.7	10.599	3.7944	0.0003	Extremely statistically significant

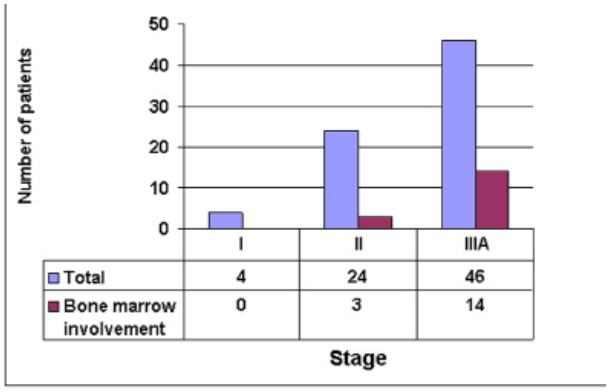

Figure 3. Chart Showing Bone Marrow Micrometastasis According to Clinical Stage

metastasis than in cases without marrow metastasis (Table 4). Mean Hb% was (9.6gm/dl) in squamous cell carcinoma with bone marrow micrometastasis and it was 10.6gm/dl in patients of squamous cell carcinoma without bone marrow micrometastasis. Average platelet count was lower among cases with bone marrow micrometastasis compared to cases without involvement of marrow in all histological types of lung cancer except in unclassified group. TLC was lower in squamous and adenocarcinoma patients with bone marrow metastasis than in those without metastasis. ESR was high in all cases with bone marrow micrometastasis than in those without metastasis. The correlation of hematological findings in patients with or without marrow micrometastasis was done by using unpaired t test (Table 5). We found no statistical significant difference in Hb% (P=0.3676), TLC (P=0.5834) and eosinophil count (P=0.4449), but obtained statistical significant difference in platelet count (P=0.0001) and ESR (P=0.0003) in analysis by Unpaired t test. We found no marrow micrometastasis in Stage I cases, 3 cases (12.5%) of micrometastasis in Stage II patients and 14 cases (30.43%) of micrometastasis in Stage IIIA patients (Figure 3).

Discussion

Bone marrow metastasis suggests advanced stage of lung cancer where treatment with curative intent is not possible. Examination of bone marrow aspirate smears should be routinely done since at times they are the only evidence of metastasis in bone marrow (Sharma et al., 2003). Aspiration and biopsy are complementary in diagnosing metastasis in marrow (Sharma et al., 2003).

Search for metastasis in non small cell lung cancer (NSCLC) is not routinely practiced unless there is suggestive manifestation. Pantel et al., 1993 reported their results on a group of 82 patients evaluated for the presence of bone marrow micrometastasis by immunohistology using monoclonal antibodies (mAb) directed against cytokeratin polypeptide CK-18. More recently, same authors presented the long-term follow-up results of a larger study population (Pantel et. al., 1996; Passlick et. al., 1999). Positive micrometastatic patients are defined as those patients who presented with at least one positive bone marrow micrometastatic cell/$0.5x10^6$ cells. The presence of two or more micrometastatic cells in the bone marrow of patients with negative nodes (PN0) was a strong predictor of recurrence and had a statistically significant impact on disease-free and overall survival (Poncelet et. al., 2001). In patients with pN1±N2, no impact either on recurrences or on survival was observed. Detection of metastatic tumours in bone marrow is of great importance for clinical staging of tumor spread because it may influence the response to treatment and the overall survival (Sharma et al., 2003). Several studies have reported shorter median survival in patients who had bone marrow metastasis than patients with extensive stage disease without marrow involvement (Poncelet et al., 2001).

In an attempt to increase the sensitivity of bone marrow examination, some studies have used immunocytochemical staining to identify micrometastasis. As facilities for IHC are not available in majority of laboratories across India, standard procedures like bone marrow aspiration, etc. were followed because of good reproducibility. It has been observed in a study conducted by a premier institute of India (Sharma et al., 2003) that bone marrow aspiration and biopsy is still relevant in detection of metastatic infiltration in bone marrow. The prevalence of bone marrow micrometastatic cells in our study is lower (23.0%, 17 out of 74 patients), than the one found by Cote et al., 1995 (40%, 17 out of 43 patients). Pantel et al., 1996 and Passlick et al., 1999 (59.7%, 82 out of 139 patients) or Oghami et al., 1997 (39%, 15 out of 39 patients). The

incidence of micrometastases was lower in our study as IHC was not available as a routine diagnostic modality apart from standard cytological and histological methods.

Over last two decades, the increasing frequency of bone marrow metastases in patients with small cell lung carcinoma (SCLC) has been documented, and SCLC is considered as a systemic disease. The incidence of bone marrow metastases in SCLC ranges from 17% to 45% during life (Hansen et. al., 1978). In our study, we get only 9 (12.2%) cases of SCLC in early stage, and 4 cases (44.4%) of marrow metastasis. Ruffato et al., 2009 documented rib bone marrow isolated tumor cells (ITC) in 17 out of 70 patients (having T1-4, N0, M0) of NSCLC. Significant survival differences were observed according to stage, presence of ITC and DNA aneuploidy (Ruffato et al., 2009). Therefore, embarking on adjuvant therapies for early-stage NSCLC based solely on the finding of bone marrow micrometastatic cells seems not justified at this point. Further follow-up of this cohort of patients might strengthen our conclusions. In our study we found marrow metastasis in 21.2% cases of NSCLC (50% for large cell carcinoma, 20% for adenocarcinoma and 17.9% for squamous cell carcinoma).

One study noted that hematological abnormalities suggestive of marrow infiltration in small cell lung carcinoma were low hemoglobin concentration and peripheral leucoerythroblastic picture (Bezwoda et.al., 1986). However these abnormalities were observed mostly in cases having clinically disseminated disease. We have not found leucoerythroblastic picture in any of our cases which comprised of patients in early stage. We found statistically significant correlation between marrow micrometastasis and decreased platelet count and increased ESR.

This study emphasizes that standard cytological and histological methods applied in this study can detect occult micrometasis in bone marrow in early stage of lung cancer which is particularly suitable for a country like India where most institutions have no IHC facility. We also highlight the necessity of detection of occult micrometastasis in early non-small lung cancer so that treatment with a curative intention can be planned.

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