Predictive Value of Clinical and Laboratory Parameters for Overt Bone Marrow Metastasis in Patients with Solid Malignancy

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Abstract

Bone marrow (BM) metastases are known to worsen the outcome in patients with malignancies and alter the choice of the treatment regimen. In an attempt to identify routine parameters for the prediction of BM metastasis we evaluated the clinical and laboratory data of 103 patients with metastatic solid tumors. We also assessed which of these factors appeared to influence survival. Three laboratory abnormalities were found to be predictive of metastasis on BM biopsy: thrombocytopenia $< 50 \times 10^9$/L OR 14.4; p = 0.02; hypocellular or dry tap aspirate OR 7.0; p = 0.02 and high serum alkaline phosphatase OR 6.5; p = 0.03. Three additional factors were found to be associated with deceased survival. Thrombocytopenia was associated with decreased survival from a median of 0.8 months to 4.1 months, p < 0.0001. Serum LDH levels more than twice the upper limit of normal were also reduced survival from 1.8 months versus 4.7 months, p = 0.03. Finally, the initial diagnosis of malignancy by bone marrow examination was associated with reduced survival from 1.8 months vs. 4.0 months, p = 0.03 in individuals where BM metastases were not the presenting feature of malignancies. Use of these simple clinical parameters may help in the early diagnosis of bone marrow metastases and improve clinical approach.

Keywords

Bone Marrow Metastasis, Predictive Factors, Survival, Solid Tumor

1. Introduction

Bone marrow (BM) metastases are diagnosed in 0.2% - 12% patients with solid malignancies. Most frequently affected are individuals with neuroblastoma or

Ewing's sarcoma, as well as those with breast, prostate and small cell lung cancer (SCLC) [1] [2] [3]. Marrow metastases are frequently indicative of metastases to other sites [4] and often predict a rapidly progressive clinical course with shortened survival which could affect the approach to treatment [2] [5]. Unfortunately, BM metastases are frequently detected only on the postmortem examination [6] [7].

The recent introduction of novel diagnostic methods for detection of disseminated tumor cells (DTC) such as reverse-transcriptase polymerase reaction (RT-PCR), fluorescent *in situ* hybridization (FISH), flow cytometry, and native or cultured bone marrow cells has greatly increased the likely detection of BM metastases [8] [9] [10] [11]. However, these techniques are not yet routinely applied or standardized. Furthermore, the prognostic impact of low-burden BM metastatic spread is still controversial [8] [12] and their effect on the therapeutic approach remains unclear [13].

Radiologic examination using computed tomography with positron emission tomography (PET-CT) or magnetic resonance imaging (MRI) the most commonly utilized noninvasive procedures for the diagnosis of BM metastasis in cancer [14]. However, these assays may not completely replace BM examination especially in cases of suspected BM involvement by malignancies of unknown primary site.

Many laboratory parameters associated with BM metastasis have been discussed in the literature but their diagnostic significance is not clearly defined [15]. These include microangiopathic hemolytic anemia, thrombocytopenia, leukocytosis, elevated red cell distribution (RDW), high serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP) uric acid and leukoerythroblastic blood picture.

Morphologic and immunohistochemical evaluation of the bone marrow by aspirate and biopsy is a readily available and technically simple procedure associated with a low rate of morbidity. It is an effective approach for the diagnosis of metastases in a cancer patient with a defined diagnosis and clinical suspicion for marrow involvement [16]. Moreover, when the primary site of the tumor is unknown, the detection and characterization of malignant cells in the BM may help to elucidate the primary source and prevent additional unnecessary diagnostic procedures [17]. Furthermore, the presence of BM metastases may be helpful in identifying hormonal receptors to help provide accurate treatment in patients whose primary tumor is unknown or inaccessible [18]. Nevertheless, marrow examination is not a routine assay in cancer patients, and the indications and diagnostic significance of these procedures have not been fully clarified.

The aim of this study was to investigate the clinical and laboratory characteristics as well as the outcome of patients with metastatic cancer who underwent bone marrow examination in our institution. We attempted to identify parameters associated with BM metastasis and risk factors which may impact survival in these individuals. We separately analyzed a subgroup of patients who were in-

itially diagnosed with malignancy by marrow examination to help elucidate the role of this procedure may play in helping to identifying the primary tumor source.

2. Materials and Methods

Results of BM biopsies and corresponding aspirates performed in our institution between June 2000 and April 2011 were retrospectively reviewed. A total 3619 BM biopsies were performed during this period of which 86 (2.4%) revealed non-hematologic tumor cells. Fourteen patients with concomitant hematological malignancy, lacking routine laboratory tests or who were lost to follow up were excluded from further analysis. These 72 individuals were divided into two groups: those patients who were referred for BM biopsy with a prior established diagnosis of metastatic tumor and in whom the biopsy confirmed BM involvement (n = 34), and individuals in whom the initial diagnosis of malignancy was the result of the BM biopsy (n = 38). As a control group we selected cases in which a prior malignancy was known but in whom BM biopsy was negative (n = 31). A final total of 103 individuals were included in this study (Figure 1).

Demographic characteristics, clinical data, location of primary tumor and additional metastases, hematological and biochemical test results, results of blood smear test, BM aspirate and trephine biopsy, as well survival time after BM examination were recorded for each individual. In general, BM aspirate and biopsy samples were obtained using routine technique from at least two different locations in the posterior superior iliac crest and occasionally from the anterior iliac spine. This was done in order to overcome limitation of unilateral examination due to focal metastatic spread (Figure 2).

The average length of the core biopsies was 1.6 cm (range 1.1 cm to 2.8 cm). Marrow biopsies were fixed in 10% buffered formalin, decalcified overnight in equal parts 10% formic acid and 8% hydrochloric acid, embedded in paraffin blocks, sectioned and initially stained with hematoxylin and eosin. Stain for pancytokeratin was performed routinely while additional immunohistochemical stains
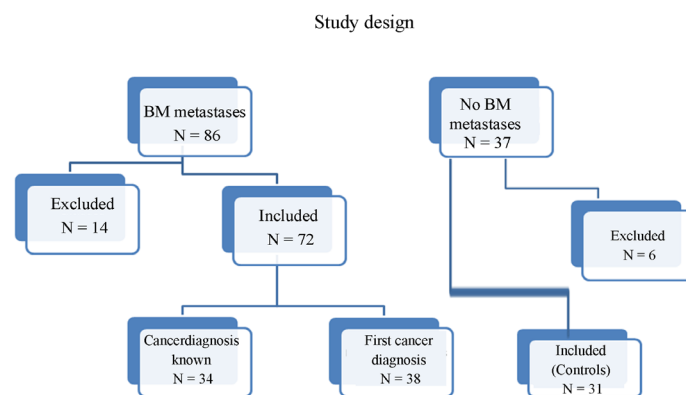
Study design

Figure 1. Study design.

A. Bone marrow aspirate from a 74 year old female with an osteolytic lesion taken from the posterior iliaccrest showing a polymorphic normocellularBM picture. (Wright-Giemsa, original magnification X400)

B. Bone marrow smear (magnification X 400) and C. (original magnification X 1000) performed simultaneously from the anterior superior iliac showing complete replacement of the hematopoietic process by clusters of large atypical cells with oval to round irregular nuclear membranes. A portion of these cells are binucleated with immature chromatin and fine basophiliccytoplasm, morphologically suggestive of breast origin
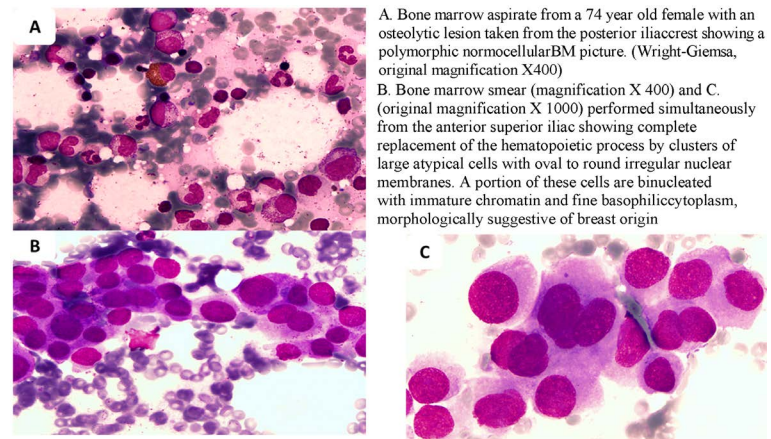
Figure 2. Metastasis.

were performed as indicated. Touch imprint from the core biopsy was prepared in 43 out of 103 (41.7%) cases including all individuals with a "dry aspiration tap". Peripheral blood and BM aspiration smears and the touch preparations were stained by Wright-Giemsa stain and at least four smears was examined under 20, 50 and 100 magnification.

This study was conducted in accordance with the Declaration of Helsinki and approved by the local ethnic committee.

3. Statistical Analysis

The SPSS 17.0 software package for Windows (SPSS Inc, Chicago, USA) was used for the statistical analysis. Basic demographic data were summarized as mean for continuous variables or n (%) for categorical parameters. The impact of different variables on predicting the presence of BM metastasis was evaluated by univariate analysis using Chi-square or Student's test. Difference between Groups 1 and 2 of $p \leq 0.05$ was considered significant. Significant variables were evaluated by multivariate logistic regression analysis. Overall survival (OS) was calculated according to the Kaplan-Meier analysis. Log-rank test was used to define significant between survival curves. OS was determinate as the period elapsed from the date bone marrow biopsy to the date of death.

4. Results

Demographic characteristics of the 72 patients with BM metastases and the 31 control cases without BM metastases are summarized in Table 1. There were no significant differences in age, male to female ratio or site of primary tumor between these groups. Of the 103 combined cases with metastatic disease, the most common primary sites of malignancy were breast (n = 25), lung (n = 23), prostate (n = 21) and gastrointestinal tract (n = 12). Cytopenia, predominantly thrombocytopenia, was the most common indication for marrow examination in 44 (61%) patients with BM metastasis, followed by bone lesions in 17 cases (38%) lymphadenopathy in 8 patients (11%), unexplained fever and hypercalcemia in 3 (4%) individuals respectively (some patients had more than one indi-

cation for biopsy). A significant difference was found in the rate of bone metastases diagnosed by imaging studies , 74% in the metastatic BM group versus 58% in controls (p = 0.008).

As summarized in Table 2, thrombocytopenia ($<140 \times 10^9$/L) was found in 40

Table 1. Demographic characteristics and primary location of metastatic solid tumors.

	Metastatic tumor patients		P value
	With BM Metastases N = 72	Without BM metastases N = 31	
Mean age (range)	67 (31 - 91)	70 (34 - 96)	0.36
Male: female ratio	1:1.1	1.8:1.1	0.13
Carcinoma type	Number (%)		
Breast	21 (29)	4 (13)	ns
Prostate	16 (22.5)	5 (16)	ns
GI tract	7 (9.7)	5 (16)	ns
Lung	(22.5)	(22.5)	
SCLC	7	3	ns
NSLSC	9	4	ns
Unknown origin	9 (12.5)	4 (13)	ns
Other	3	6	
Other metastases			
Brain	10 (14%)	4 (13)	ns
Lung, liver, adrenal	31 (43)	13 (42)	ns
Bone*	53 (74)	18 (58)	0.008

*Suspected by imaging: x-ray, CT, MRI or bone scan.

Table 2. Laboratory parameters of 103 patients with metastatic malignancy.

Characteristic (normal range)	Patient with Bone marrow metastasis (n = 72)	Without Bone marrow metastasis (n = 31)	P value
Hb mean g/dL (range) Normal range (12 - 15 g/dL)	10.3 (3.5 - 14.6)	11.2 ± 1.86	**0.023**
<12g/dL incidence %	83.3%	61.3%	**0.015**
White blood cell, mean (range)) Normal range (4.5 - 11/ $\times 10^9$)	9.3 (1.2 - 89.6)	7.8 (1.28 - 15.9)	0.43
PLT count, mean $\times 10^9$/L	159 (4 - 570)	264 (27 - 486)	**<0.001**
PLT $<140 \times 10^9$/L, incidence	55.6%	16%	**<0.001**
PLT $< 50 \times 10^9$/L, incidence	26.4%	3.2%	**0.006**
MCV, mean fl (range)	86.2 (66 - 109)	86.9 (80 - 101)	0.5
RDW, mean (range %)	16.6 (13 - 26)	16.0 (13 - 25)	0.2
MPV, mean fl (range)	8.73 (7 - 12)	8.17 (6.3 - 9.5)	**0.03**
Albumin, mean g/dL (range)	3.4 (2.3 - 4.6)	3.2 (1.9 - 4.8)	0.35
Calcium, mean g/dL	8.97 (6.8 - 11.2)	8.9 (7.2 - 10.2)	0.6
LDH, mean U/L	1515 (234 - 9200)	992 (306 - 6465)	0.12
>2 ULN (480 U/L), incidence	50%	16.7%	**0.002**
Alkaline phosphatase, mean U/L	283 (51 - 1086)	132 (46 - 701)	**<0.001**
>2 ULN (104 U/L), incidence	35.7%	9.7	**0.007**
Ferritin ng/mL, mean (range)	1759 (220 - 4480)	254 (3.7 - 650)	0.007
Hypoplastic/dry tap BM aspirate	58.8%	16.6	0.001

of 72 patients (55.6%) with BM spread compared to 5 of 31 (16%) without this finding (p < 0.001). Furthermore, severe thrombocytopenia (<50 × 10^9/L) was noted in 19 patients with BM metastases (26.4%) as compared to only one individual in the no metastases group (3.2%; p = 0.006). Mean platelet volume was also found to be significantly different measuring an average of 8.73 fl in patients with metastatic marrow involvement and 8.17 fl in those without (p = 0.014).

Anemia defined as hemoglobin < 12 g/dL was noted significantly more often in patients with metastatic marrow (n = 60, 83%) versus non-affected individuals (n = 19; 61%) (p = 0.015). Leukoerythroblastic picture in the blood smear was observed in 28 of 34 (82.4%) patients with BM involvement and in no one without (p < 0.001). Elevation of serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels more than two times the upper limit of normal (UNL) was seen in a significantly higher percentage of patients with marrow involvement than without: 50% versus 16%; p = 0.002 for LDH and 35% versus 9%; p = 0.007 for ALP. Finally, mean serum ferritin level was noted to be significantly higher in patients with marrow metastasis than unaffected individuals (1760 ng/ml versus 255 ng/ml; p = 0.027).

Aside from the direct finding of tumor cells in the aspirate, multivariate analysis revealed three additional factors which were associated with the presence of metastases on BM biopsy: thrombocytopenia <50 × 10^9/L (OR 14.4; 95% CI, 1.62 - 129; p = 0.02); hypocellular/ dry tap aspirate (OR 7.0; 95% CI, 2 - 23.6; p = 0.02) and elevated serum alkaline phosphatase (OR 6.5; 95% CI, 1.2 - 35.3; p = 0.03). In contrast, elevated serum LDH was not found to be significantly correlated with BM spread (OR 1.36; 95% CI, 0.32 - 5.7; p = 0.2).

Cytological examination of BM aspirate recognized malignant cells in 46 out of 60 (76.6%) patients. Marrow involvement by aspirate was diagnosed in 13 of 14 patients (93%) with prostate carcinoma, 13 of 18 (%) with breast, 9 of 15 (%) with lung, 3 of 4 with gastrointestinal, and 5 out of 6 patients with malignancy of unknown origin. Hypocellular or dry tap marrow aspirate was found in 35 out of 60 (58.8%) patients with BM involvement and in only 5 of 30 patients (14%) without (p = 0.001). Of the 38 patients in whom the diagnosis of cancer was initially established by marrow biopsy, 29 (76.3%) had the primary tumor site established during further clinical investigation. Of interest, pathologic study alone was sufficient to establish the primary tumor in approximately half of these individuals, most commonly prostate (7 of 8) and lung (10 of 14) primaries.

We further compared the clinical characteristics of patients who had a prior malignancy diagnosis who were referred for staging or with an unrelated clinical indication for marrow biopsy, with those individuals in whom malignancy was initially diagnosed by the marrow examination. The group with cancer diagnosed by BM biopsy had similar mean age (p = 0.14) but a higher proportion of males (70%, p = 0.009), more frequently suffered from severe thrombocytopenia and had a higher incidence of small cell lung cancer (n = 9, 23.6% vs. n = 2, 6%, p = 0.03).

All 103 patients died, but median survival for patients with marrow involvement was significantly shorter than for those without (2.2 versus 5.1 months; p =

0.023). We separately analyzed risk factors associated with patient survival with metastatic BM involvement. For severe thrombocytopenia median survival was reduced to 0.8 months (95% CI, 0.1 - 1.5) versus 4.1 months for unaffected individuals (95% CI 2.0 - 6.1); p < 0.0001) (**Figure 3**). Elevated serum LDH was similarly associated with decreased survival from a mean of 1.8 months (95% CI; 0.5 - 3.0) versus 4.7 months in those with normal serum levels (95% CI; 3.0 - 6.3, p = 0.03). The initial diagnosis of malignancy by bone marrow examination also was a marker for decreased survival to 1.8 months (95% CI; 0.3 - 3.3) from a baseline

(a)

(b)

(c)
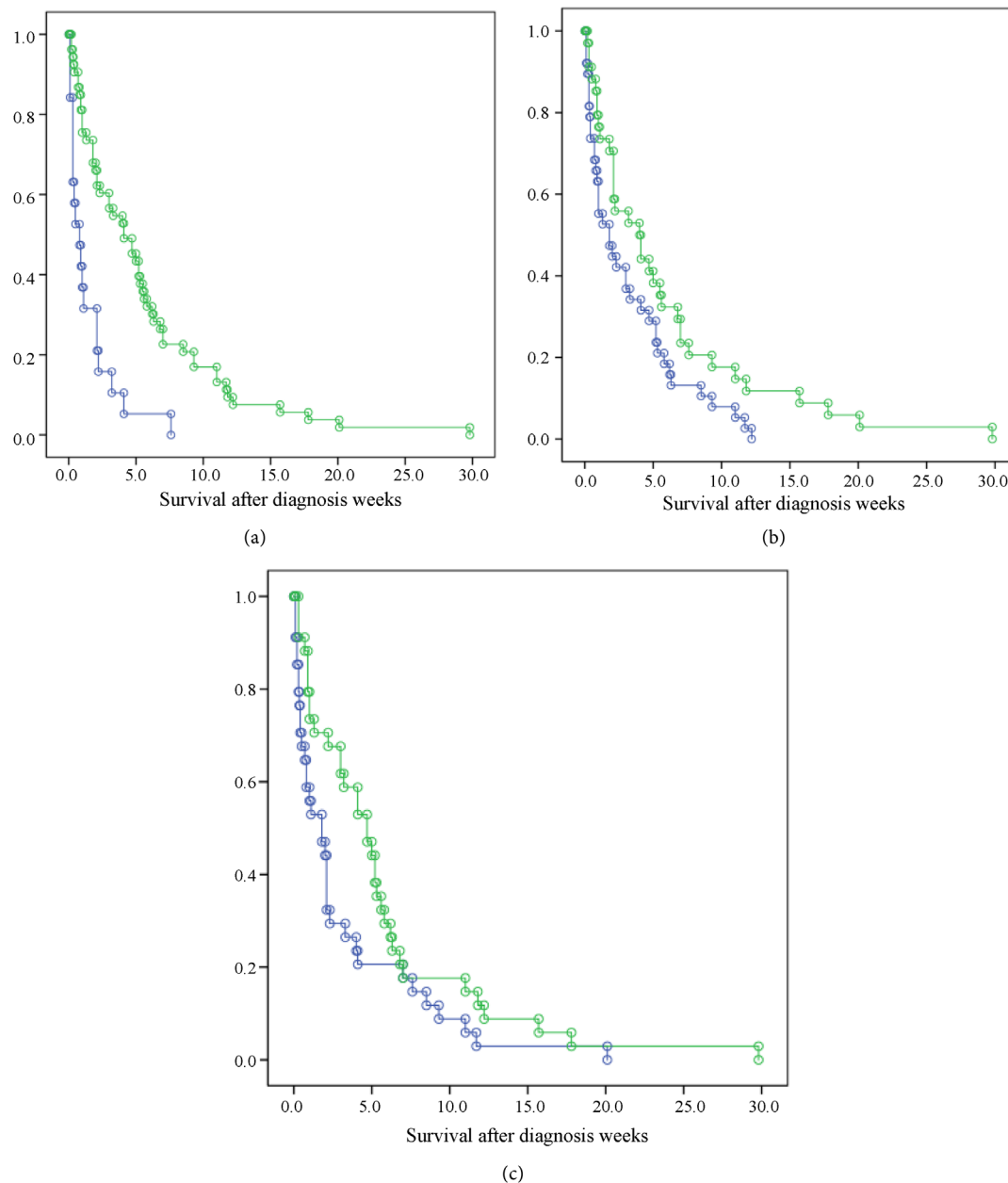
Figure 3. (a) Median Survival of patients (weeks) after diagnosis BM metastasis with PLT > 50 k/Ul (green line) 4.1 months versus PLT < 50 k/uL (blue line) 0.8 months, p < 0.001; (b) Median survival of patients with previously known metastatic cancer 4.0 m. vs initially diagnosed with malignancy by BM examination 1.8 m, p = 0.03; (c) Median survival according to LDH level < 2ULN (upper limit of normal) 4.7 m. vs. LDH > 2ULN 1.8 m, p = 0.043.

of 4.0 months if the diagnosis was established by other means (95% CI; 1.3 - 6.7, p = 0.03).

5. Discussion

Metastatic BM involvement has a known negative effect on the clinical course of malignancy, and may alter choice of the treatment regimen because of limited hematopoietic reserve and worsened patient outcome. However, detection of BM metastases may be helpful to identify hormonal receptors to help provide accurate treatment in patients whose primary tumor is unknown or inaccessible [18].

In this study we evaluated the efficacy and prognostic significance of BM examination in patients with metastatic cancer, and analyzed the clinical and laboratory characteristics of this patient population. In this 10 year retrospective review we identified 103 individuals with metastatic malignancy in which 72 had marrow infiltration by biopsy. As in other studies a higher incidence was observed in patients affected with breast, lung and prostate cancer. BM metastases occurred not only in patients with known disseminated carcinomas, but also as the presenting clinical manifestation. In over half of our patients the primary localization of their solid tumor was diagnosed by BM examination, a relatively high proportion when compared with other recent publications [19].

As in other published studies [20] [21] BM involvement in our patient cohort was associated with a significant reduction in survival: 2.2 months compared to 5.1 months in those without such metastases (p = 0.023). Particularly short survival of 0.8 months was noted in patients with severe thrombocytopenia ($<50 \times 10^9$/L) confirming previously published reports [22] [23]. Severely reduced platelet counts are felt to decrease patient tolerance to antitumor therapy and increase the incidence of fatal bleeding complications. High LDH level also predicted a poor outcome for patient with BM metastasis (median survival 1.8 months) probably reflecting a high burden of BM metastasis. Individuals whose primary tumor was only diagnosed by bone biopsy also were found to have shorter survival (1.8 months) most likely caused by the delay in diagnosis and resultant therapy decisions.

Despite the potential significance of this complication on the management of patients with malignancy, BM examination is not included in a list of routine assessments. In clinical practice referral for BM biopsy is usually reserved for those patients who develop a significant hematologic abnormality, mainly cytopenia. However, hematologic manifestations in malignant disease may have many different causes making it a priority to define which parameters are likely to be associated with marrow metastases, expediting diagnosis and preventing unnecessary procedures. Several prior studies have also addressed this issue and attempted to evaluate potential clinical indicators [15] [19] [22] [24] [25] [26] [27].

Multivariate analysis of our patient population identified three factors that were correlated with the presence of bone marrow infiltration: thrombocytope-

nia $< 50 \times 10^9$/L (OR 14.4), hypoplastic or dry tap aspirate (OR 7.0) and high alkaline phosphatase (OR 6.5). Peripheral blood leukoerythroblastic picture (characterized by nucleated and tear drop red cells and immature myeloid precursors) has been proposed previously as a sensitive indicator of BM involvement, described in 19.8% to 79% of patients, appearing most frequently in cases of gastrointestinal, prostate and breast cancer, as well as those associated with medullar fibrosis [20] [28] [29] [30] [31] [32]. In our cohort 82% of patients were seen with this finding perhaps reflecting a late stage of diagnosis. We also found a strong correlation (91.7%) between dry tap or hypocellular BM aspiration and leukoerythroblastosis in our series. This finding was not included in the final analysis of predictive factors because of the small number of recorded blood smears. However, we consider a leukoerythroblastic picture an important but late sign to the presence underlying metastatic marrow disease.

In contrast to prior reports showing association of low MPV index and BM involvement [33] [34], many of our patients with metastatic BM had a high MPV.

Also in contrast to other published studies [35] [36] our results demonstrated usefulness of BM aspirate examination for detection of cancer cells (72% cases) with very high rate (93%) of diagnosis a potential treatable prostate carcinoma.

6. Conclusion

In conclusion, patients with BM metastases have very poor prognosis, particularly those individuals in which the initial diagnosis of malignancy was established on the bone biopsy. Additional poor prognostic factors in these patients include thrombocytopenia and elevated LDH which also predict a shortened survival. Hypoplastic BM aspirate, thrombocytopenia and high serum alkaline phosphatase were associated with BM involvement in patients with metastatic malignancy and may be indicators of the need to perform this procedure in previously undiagnosed cases. Based on these findings, we propose that these variables be followed in patients with malignancies, and in the absence of other evident causes (drug, DIC, etc.), a BM examination be performed. Accurate, prompt diagnosis of primary solid tumor via BM biopsy may prevent the need for additional invasive procedures and ultimately help in forming an appropriate treatment approach focused on potential treatable primary carcinoma.

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