Am J Blood Res 2023;13(1):1-11
www.AJBlood.us /ISSN:2160-1992/AJBR0145690

Review Article

Cytomorphological evaluation of non-haematopoietic malignancies metastasizing to the bone marrow

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Received August 2, 2022; Accepted December 25, 2022; Epub February 15, 2023; Published February 28, 2023

Abstract: Bone marrow (BM) is one of the rare but important site of metastasis of solid tumors. The key steps of metastasis include invasion, intravasation, circulation, extravasation, and colonization. Tumor cells may express some adhesion molecules that promote the transmigration to the marrow space and link them to the marrow stroma with subsequent engraftment. It is important to detect the bone marrow metastasis for initial clinical staging, therapeutic selection, prognostic risk stratification, assessment of response to therapy and predicting relapse. Prognosis of non-hematopoietic malignancies with BM metastasis is dismal. Due to occulting and atypical clinical manifestations, bone marrow metastases can be easily missed or misdiagnosed, leading to higher mortality rates. The important factors on which the prognosis of patients with bone marrow metastases depends are primary tumor site, performance status, platelet count, and therapeutic regimens (systemic chemotherapy or palliative/supportive care). Further, in cases with BM metastasis with unknown primary sites, misdiagnosis can lead to delayed initiation of therapy and increased mortality. BM metastasis is seen in less than 10% of patients with metastatic cancer and is common in lung, breast or prostate carcinoma. Bone marrow metastasis can be presented as the initial presentation with hematological changes and may be misdiagnosed as a primary haematopoietic disorder. Leucoerythoblastic blood picture is the most common peripheral blood smear finding indicating BM metastasis, may be an indicator of associated BM fibrosis. Bone marrow aspiration and biopsy with immunohistochemistry (IHC) is an easy, cost effective and gold standard method of detection of BM metastasis. BM biopsy is superior to bone marrow aspirate for detection of metastasis. Morphology of metastatic cells is as per the primary site of tumor. Immunohistochemistry is a useful adjunct to morphology in reaching a definitive diagnosis even in case with carcinoma unknown primary (CUP) and also in diagnosing case of unsuspected malignancies. Though bone marrow is not among the most common site of involvement in CUP, which are liver, bone, lymph nodes and lung. But BM, if involved, the site of origin is determined using the immunohistochemistry panel applied to the metastatic deposits based on the morphology The aim of the review is to discuss the hematological findings of non-haematopoietic malignancies metastasizing to the bone marrow, emphasizing on histomorphology with IHC and its significance in establishing primary diagnosis in clinically unsuspected cases.

Keywords: Bone marrow metastasis, bone marrow biopsy, immunohistochemistry, carcinoma of unknown primary

Introduction

Bone marrow (BM) is one among of the rare but important site of metastasis of non-hematopoietic solid malignancies and occurs in less than 10% of patients with metastatic disease. It is associated with a poor prognosis. BM metastasis is common in patients with lung, breast and prostate carcinoma. Clinical findings were highly variable, consistent with the underlying disease. Common systemic presentation of BM metastasis are bone pain, spinal cord compression and pathological fractures [1]. Common biochemical findings are elevated lactate dehydrogenase, hypercalcemia, hypoproteinemia; And elevated serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels [2]. In most cases, the growth of metastatic carcinoma in the BM leads to trabecular bone destruction, resulting in osteolytic lesions. Some carcinomas may stimulate the active new woven bone formation, producing osteosclerotic lesions and a few may have a mixed lytic-

sclerotic pattern depending on phase; Sclerotic in late phase. Bone marrow metastasis can be the first presentation and may be presented as cytopenias simulating a primary haematopoietic disorder. There are a number of findings in the peripheral blood like microangiopathic hemolytic anemia, leukoerythroblastic blood picture and cytopenias (mostly anemia and thrombocytopenia) which are indicators of bone marrow metastasis. Very rarely circulating tumor cells can be identified on peripheral blood examination. However, in most of the patients with bone marrow infiltration, there is no significant peripheral blood findings [3]. Bone marrow aspiration/biopsy with immunohistochemistry (IHC) is required for a definitive opinion. It is important to detect the bone marrow metastasis for initial clinical staging, therapy selection, prognostic risk stratification, assessment of response to therapy and predicting relapse. Further, bone marrow examination has another advantage in those cases where patients without a clinically detectable primary site of origin, but presented with symptoms of malignancy or pathological fractures. In these cases, histo-morphological study helps in assessment of the primary site of non-hematopoietic solid malignancy.

Bone marrow aspiration is a simple, rapid and cheap method for detection of bone marrow metastasis. Bone marrow biopsy with immunohistochemical and/or molecular techniques (as polymerase chain reaction) may be required to detect bone marrow metastasis in case of microscopical tumor burden. Detection of BM metastasis has both therapeutic and prognostic significance. Histomorphology along with immunohistochemistry is a useful adjunct in reaching a definitive diagnosis of cases unsuspected for malignancy or even in cases with unknown primary. The aim of the review is to discuss the hematological findings of non-haematopoietic malignancies metastasizing to the bone marrow, emphasizing on histomorphology with IHC and its significance in establishing primary diagnosis in clinically unsuspected cases.

Prognostic significance of bone marrow metastasis

Bone marrow metastasis affects the stage of the malignancy as well as the time of survival. Metastatic cancer is a therapeutic challenge to clinicians, especially for patients with bone marrow metastases. Patients with bone marrow metastases have a dismal prognosis due to a rapid disease progression and a poor response to treatment [4, 5]. Variable median survival time was reported across the literature. The factors on which the prognosis of patients with bone marrow metastases depends are primary tumor site, performance status, platelet count, and therapeutic regimens (systemic chemotherapy or palliative/supportive care) [6, 7]. Zhou et al. [8] described a median survival of 3 months in patients with bone marrow metastasis with median survival time of 8 months in the patients with ECOG PS of 0 or 1 (range, 1-82 months) and 1 month (range, 0.5-15 months) in the patients with ECOG PS of 2 or 3. Patients with primary cancers of unknown origin and patients with best supportive care survived significantly shorter than the other group of patients with the known origin and patients with systemic therapy, respectively, with median survival of 1 month in both groups. Kucukzeybek et al. [9] described a lower median overall survival after bone marrow metastasis which was 28 days with no statistically significant difference between patients with primary known and unknown tumor. But there was statistical difference between the survivals with type of primary tumor, e.g. breast cancer with BM involvement had longer survival than gastric cancer with BM involvement. The median overall survival difference was statistically significant between patients who have anemia and thrombocytopenia. Median overall survival times showed significant differences between chemotherapy applicable and nonapplicable patients. Kwon et al. [10] evaluated 26 patients with bone marrow metastasis and found the median overall survival as 37 days with significant differences between chemotherapy applicable and nonapplicable patients.

Mechanism of BM metastasis

The key steps of metastasis include invasion, intravasation, circulation, extravasation, and colonization. Tumor cells may express some adhesion molecules that promote the transmigration to the marrow space and link them to the marrow stroma with subsequent engraftment [11]. Signaling pathway involving stromal derived factor 1 (SDF-1)-CXCR-4 induces BM invasion and homing of tumor cells. Homing factors located on malignant cells are Intigrins,

cadherins, E- and P-selectin and the corresponding molecules located on the stromal cells are cadherin-11, vascular cell adhesion molecule, fibronectin and selectin. Some extracellular matrix proteins also involved are laminin, type IV collagen, osteopontin, sialoprotein and metaloprotienases [12]. Studies also suggested, BM derived cells expressing vascular endothelial grown factor 1 also create a niche for metastasis [13]. After metastasis, BM microenvironment help in growth and proliferation. Factors affecting tumor proliferation are mainly insulin like growth factor-1 and transforming growth factor β present in osteoid which increased proliferation and reduce apoptosis. Other cytokines that increase proliferation and survival of tumor cells are fibroblast growth factor 1 and 2, and platelet derived growth factor [12]. In adults, the tumors that most commonly metastasize to the marrow are carcinomas of the prostate, breast, gastrointestinal tract, lung, thyroid and kidney and in children they are neuroblastoma, rhabdomyosarcoma, Ewing tumor and retinoblastoma. After the tumor has progressed to locally invade the vessels, few disseminated tumor cells are released and evade the immune system, circulating from the primary tumor site to the bone marrow. The tumor cells start colonizing the bone marrow microenvironment and some adapt to the local environment. Specific organ microenvironment recruits and supports the survival and growth of specific types of cancer cells. For example, the osteotropism feature of breast cancer suggests the presence of specific factors from the bone that activate the recruitment of breast cancer cells to the bone marrow [14]. Some types of bone marrow cells have been evaluated for their contribution to attracting breast cancer cells, such as osteoblasts, osteoclasts, and adipocytes. These studies report that some adipocytokines are involved in breast cancer osteotropism, including CXCL12, RANKL, leptin, and IL-1β [15, 16]. Adipocytokines not only are associated with the establishment of a pro-tumor microenvironment and organ-directed metastasis but also mediate disease progression, favoring the growth and proliferation of tumor cells [17].

Methods of detection of BM metastasis

Different methods to detect BM metastasis are: Radiological investigations, bone marrow aspiration/biopsy with IHC, tumor biomarkers or molecular techniques.

Radiological studies

Radiological evaluation including computed tomography (CT), whole body magnetic resonance imaging (MRI) or 18 F-fluorodeoxyglucose positron-emission tomography (FDG-PET) are the commonly used methods to detect BM metastasis. CT is not able to detect early BM metastasis. PET-CT have a high ability to detect bone marrow involvement in metastatic disease by means of increased FDG uptake in growing metastatic cancer cells [18]. Rarely, BM metastases presenting with symmetrical diffuse BM involvement on radiological evaluation, may be misdiagnosed with leukemia [2]. This can be attributed to presence of small primary tumors that do not have overt clinical symptoms at their primary sites. These procedures are available only at advanced medical centers in cities and are expensive too.

Peripheral blood findings

Common hematological alteration associated in cases with bone marrow metastasis are cytopenias, commonly; normocytic normochromic anaemia, neutropenia or thrombocytopenia. The bone marrow metastasis affects the normal haemopoiesis leading to myelopthesic anaemia and other cytopenias [19]. Anaemia is mostly normocytic anemia of chronic disease. Microcytic iron deficiency anemia or hemolytic anaemia (microangiopathic hemolytic anemia) may be present in few cases [12, 20]. Leucoerythoblastic blood picture is the most common peripheral blood smear finding indication BM metastasis, may be an indicator of associated BM fibrosis. The mechanism of leucoerythroblastic blood picture is not clearly understood. BM invasion by metastatic tumor cells may cause early release of some cytokines (eg. granulocyte colony stimulating factor), leading to the development of a myelophthisic blood picture with presence of nucleated RBCs and immature myeloid precursors in peripheral blood [3]. Some studies suggest elevated RDW, low MPV has a significant predictive value for BM metastasis [21, 22]. Occasional cases show circulation malignant cells on peripheral blood smear examination. Circulating tumor cells have been described in cases of breast carcinoma, small cell carcino-

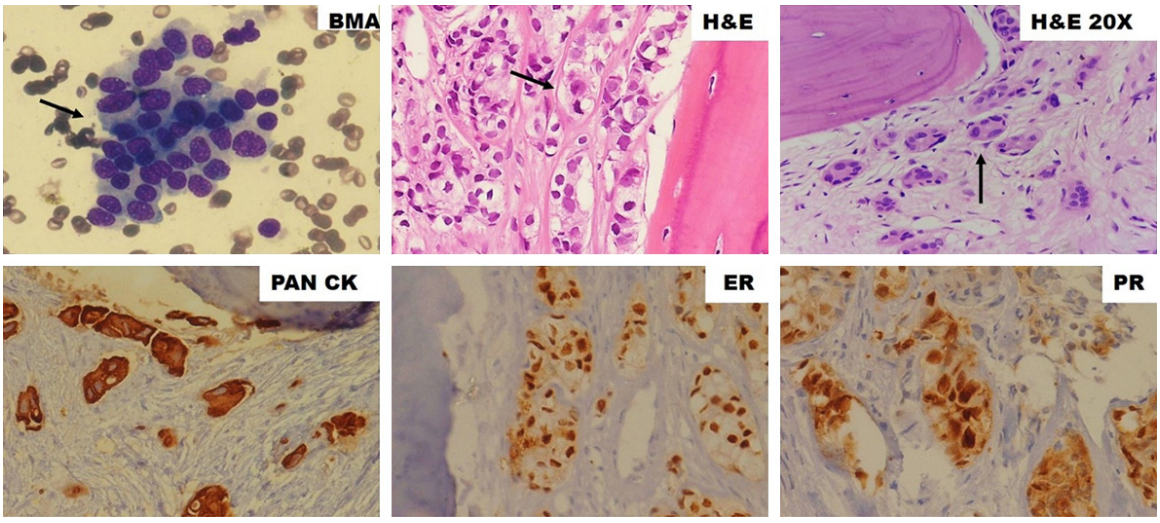

Figure 1. BM aspirate and biopsy with IHC findings of a case of ductal carcinoma of breast.

ma, ocarian carcinoma, neroblastoma and rhabdomyosarcoma; and sometimes mimicking acute leukemia [23].

Bone marrow aspiration findings

BM aspiration is an easy and cheap method for detection of BM metastasis by solid tumors. Early diagnosis of BM metastasis is possible by examining bilateral BM and combining meticulous search of metastatic tumor cells on BM aspirate as well as imprint smears. These procedures will avoid the unnecessary delay due to decalcification and IHC for BM biopsy results. Bone marrow aspirates are also helpful in cases with inadequate/fragmented bone marrow biopsies. Examination of bone marrow touch preparation is also crucial as in many cases tumor cells are present only on touch preparation, because of extensive bone marrow fibrosis [24]. Distribution of cells in BMA may be a. scattered single cell, b. acinar/rosette formation, c. small clusters, d. large sheets of tumor cell, e. near total replacement by tumor cells. Cell morphology varied from small round cells to large bizarre looking anaplastic cell to multinucleated cells. Degenerated neoplastic cells may be seen at the edge of BM smears. BM metastasis by small round cell tumors as neuroblastoma, retinoblastoma or Ewings sarcoma/PNET consists of malignant round cells that are slightly larger or double the size of red blood cells with bluish cytoplasm which may present singly or in sheets or clusters. Adenocarcinomas contributed the majority of metastatic BM lesions. Morphology of metastatic cells are as per the primary site of tumor. Gastrointestinal tumor metastasizing to BM has abundant cytoplasm and large vesicular nuclei, present in loose clusters and sheets. Metachromatic cytoplasmic granules may be present in some cases. BM metastasis from a case of ductal carcinoma breast shows cohesive clusters of cells with moderately pleomorphic overlapping nuclei. **Figure 1** showed a metastatic breast carcinoma BM aspirate and biopsy with IHC findings. Metastatic germ cell tumor cells has clusters of moderately pleomorphic cells with discernible cytoplasm. Adenocarcinoma of lung shows clusters of tumor cells, however, there may also be individual cells or acinar/glandular arrangements, with high nucleo-cytoplasmic ratio, irregular nuclear membrane, granular chromatin and prominent nucleoli. **Figure 2** showed metastatic adenocarcinoma of lung with BM aspirate and biopsy with IHC findings. Small cell carcinoma of lung shows tight clusters of hyperchromatic cells with scanty cytoplasm, nuclear molding with salt and pepper like chromatin. Small cell prostate carcinoma is a rare form of extrapulmonary high-grade neuroendocrine carcinoma with similar morphological features. **Figure 3** showed BM aspirate and biopsy with IHC findings of a case of neuroendocrine carcinoma of prostate. Morphology of metastatic squamous cell carcinoma depends on the degree of differentiation; predominantly shows sheets, clus-

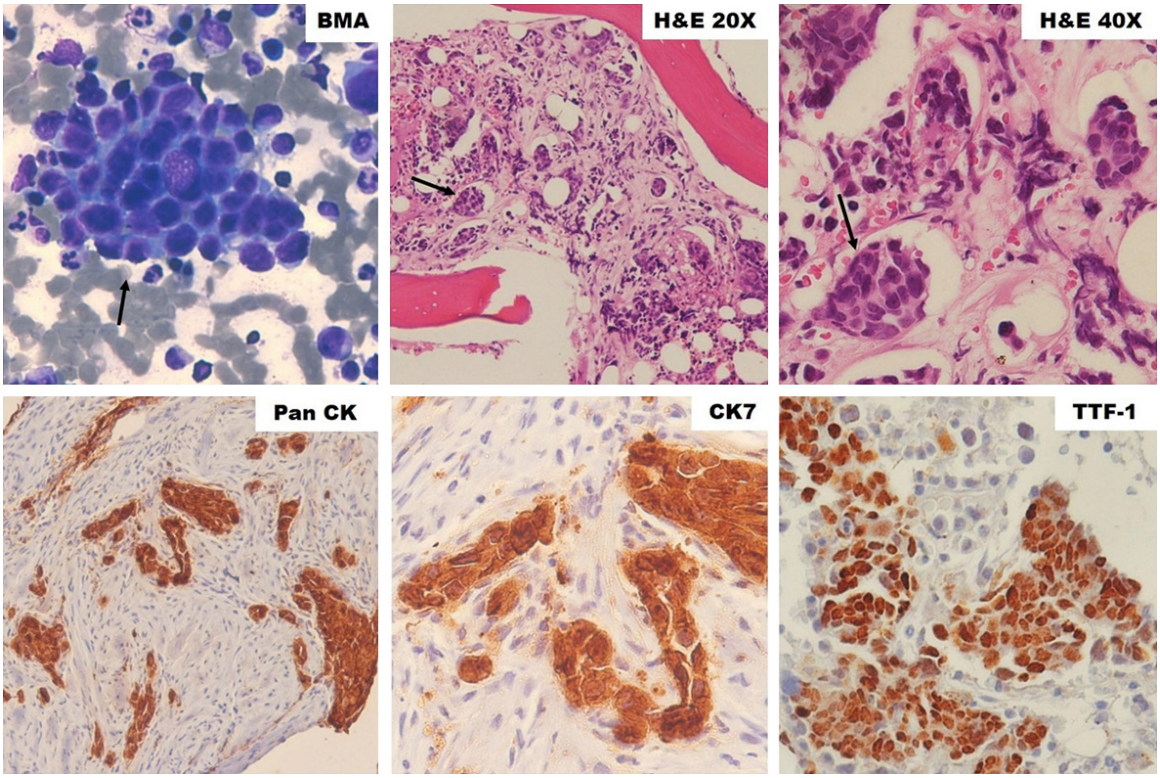

Figure 2. BM aspirate and biopsy with IHC findings of a case of adenocarcinoma lung.

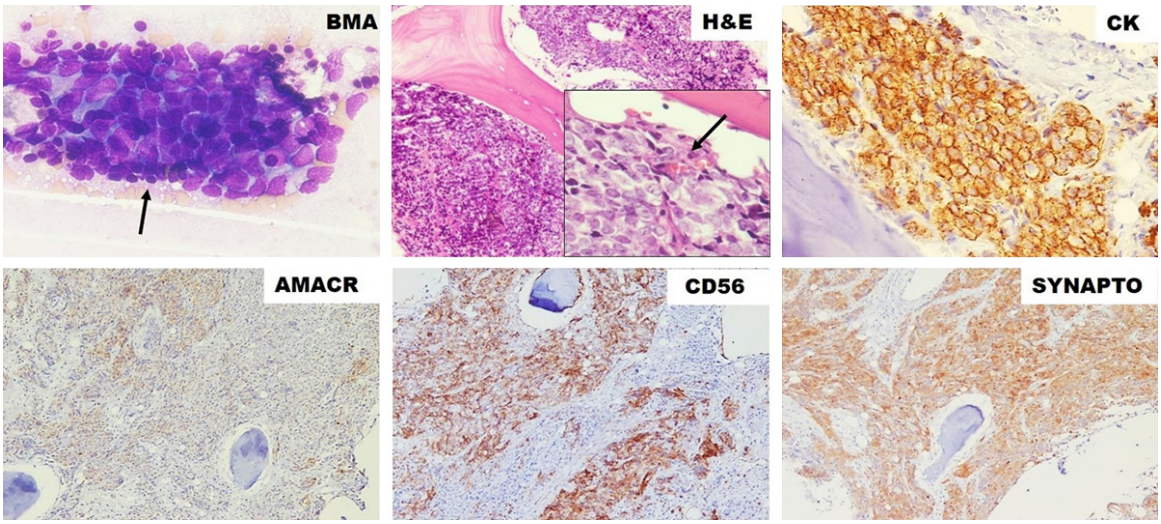

Figure 3. BM aspirate and biopsy with IHC findings of a case of neuroendocrine carcinoma of prostate.

ters, and scattered malignant cells with moderate anaplasia, hyperchromatic nuclei, and scanty dense cytoplasm. **Figure 4** showed metastatic squamous cell carcinoma of buccal, cavity with BM biopsy with IHC findings. Hepatocellular carcinoma is characterized by abundant eosinophilic cytoplasm, polygonal shape and large vesicular nuclei with prominent central nucleoli. Some of the tumor cells may arranged in sheets and clusters with acinar pattern or in trabecular pattern. Eosinophilic intranuclear inclusions may also be noted. Pancreatico-billiary carcinoma metastasizing to bone maroow showed malignant cells with

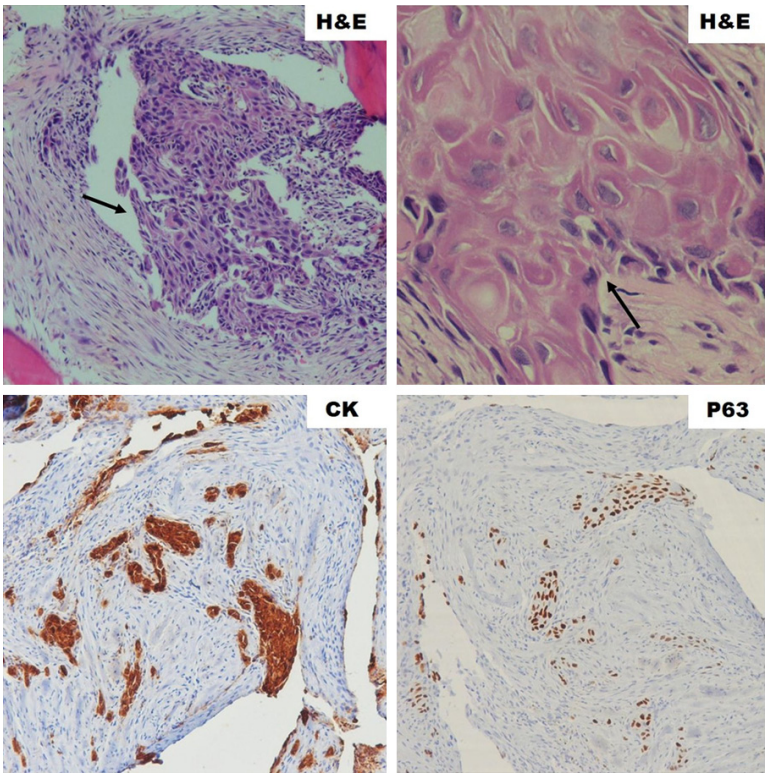

Figure 4. BM biopsy with IHC findings of a case of squamous cell carcinoma metastasizing to BM.

glandular differentiation, significant nuclear pleomorphism, crowding, 3-dimensional clustering, and prominent nucleoli. **Figure 5** showed BM aspirate with biopsy findings of a case of pancreatico-billiary carcinoma metastasizing to BM. Adenosquamous carcinoma had sheets of adenocarcinoma cells characterized by mildly pleomorphic cells with a moderate amount of cytoplasm and discretely present malignant squamous cells with hyperchromatic nuclei and abundant glassy to blue cytoplasm. Some of the adenocarcinomatous cells are tightly pressed against each other with vacuolated cytoplasm and complex nuclear features. Nucleoli are prominent in high-grade tumors. BM aspirate is normocellular to hypo or hypercellular. It may be hemodilute due to presence of marrow desmoplasia. Morphological assessment BM smears has high inter-operator variation. It is even more challenging to identify occult metastatic cancer cells manually because they are not distributed evenly across the smear. Most of them are located at the edges of slides beyond for recognition, leading to misdiagnosis in clinical practice. Poorly differentiated carcinomas are difficult to differentiate from hematopoietic tumors, particularly acute leukemia or large cells lymphoma.

Bone marrow biopsy findings

BM biopsy is more superior to bone marrow aspirate for detection of metastasis. IHC can further helpful in definite diagnosis, identification of primary site of solid malignancy and diagnosing dual malignancies infiltration to BM. Distribution of metastatic tumor cells in bone marrow biopsies is categorized as a-single cells or small clusters, b-multiple tumor clusters, c-large tumor masses with few preserved marrow spaces and d-total marrow replacement by the metastatic tumor cells. In the absence of neoplastic cells in the marrow, infiltration is suspected by other features like necrosis, desmoplasia, inflammation, osseous metaplasia and granulomas [22]. Desmoplasia and osseous metaplasia may be found in few cases resulting in dry tap. Apart from staging in a known malignancy, recognition of tumor in random biopsies impose challenge to the pathologist in finding the unknown primary. The use of basic laboratory tests, imaging studies, IHC and serum tumor markers is highly suggestive in identification of unknown primary [25].

The most common tumor metastasizing to BM is neuroblastoma and gastrointestinal tumor in children and adult, respectively [26]. Few studies suggested that prostatic adenocarcinoma is the most common solid tumor in adult metastasizing to BM [27]. Other common tumors metastasizing to bone marrow are carcinoma of the breast and lung in adults and Ewing's sarcoma, rhabdomyosarcoma, and retinoblastoma in paediatric age groups [2, 28]. The various metastatic adenocarcinomas have the same histomorphology so it becomes difficult to define the origin in cases of unknown primary tumors. In those cases immunohistochemistry plays the pivotal role in identifying and confirming the primary site of bone marrow

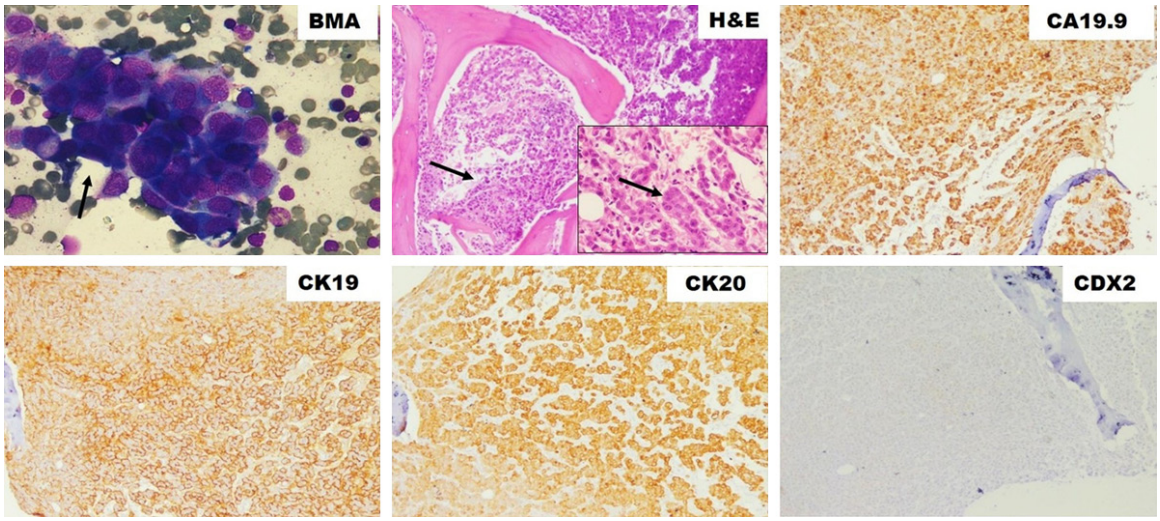

Figure 5. BM aspirate and biopsy with IHC findings of a case of pancreatico-billiary carcinoma.

metastasis. Once the morphology is identified, various immunohistochemistry markers are used to evaluate the tumor. The small round cell tumors comprise of the few most aggressive solid tumors present predominantly during childhood and adolescence and therefore accurate diagnosis is critical for the management of these patients. The neoplasms include Ewing's sarcoma, primitive neuroectodermal tumor, retinoblastoma, neuroblastoma, rhabdomyosarcoma, mesenchymal chondrosarcoma, small cell osteosarcoma and non-hodgkins lymphoma. The bone is the common site of metastasis for these tumors and its presence indicates an increased risk for subsequent distant metastasis and poor prognosis. Frequency of BM involvement by small round cell tumor may varies between 10 to 20% [29]. In a study done by Asif et al. the frequency of bone marrow infiltration by small round cell tumors excluding lymphomas was 19.5% [30]. The histomorphology of all the small round cell tumors show primitive round blue cells which becomes difficult to locate on bone marrow biopsy when present in singles. Immunohistochemistry plays a crucial role in identification and categorization of these tumors.

Carcinoma of unknown primary

Bone marrow is the important site of metastasis and its detection has a major role in prognosis and management of the patient. In clinically unsuspected cases where primary site is unknown, the morphological findings of the metastatic tumor along with immunohistochemistry is valuable in elucidating the primary site of tumor. Cancer of unknown primary (CUP) accounts for 3 to 5% of all cancers and is defined as heterogenous group of tumors presenting as metastasis with unidentifiable primary site despite clinical history, physical examination, radiological and biochemical investigations [31]. Though bone marrow is not among the most common site of involvement in CUP which are liver, bone, lymph node and lung. But BM, if involved, immunohistochemistry plays the pivotal role in diagnosing the CUP in bone marrow metastasis. The site of origin is determined using the immunohistochemistry panel applied to the metastatic deposits based on the morphology [22]. Initial diagnostic IHC panel include a few antibodies directed against epithelial antigens (pan-Cytokeratin Plus [AE1/AE3+8/18]), lymphoid antigens (CD45 or CD-20 and CD3) and melanocyte-differentiation antigens (S100 protein, SOX10) [32]. Vimentin is a nonspecific marker; however, a vimentin can be used as vimentin negative malignancy is unlikely to be a sarcoma (with the exception of alveolar soft part sarcoma), lymphoma, or melanoma [33]. IHC used in undifferentiated carcinoma are Pan CK, EMA, Chromogranin, LCA, S100. Pleomorphic sarcoma can be initially differentiated on Vimentin, Desmin, SMA, CD117, S100, CD99, CD68. IHC for differential diagnosis of round cell tumor are Pan CK, Vimentin, Desmin, CD99, NSE, LCA, Chromogranin [22]. Further specific IHC can be used

Table 1. Features of common solid malignancies metastasizing to bone marrow

Site	Common sites of metastasis	IHC
Lung	CNS, bone, liver, respiratory system	Adenocarcinoma: CK7+, TTF-1+, Napsin-1+
		SCC: CK 5/6, p40
Breast	Bone, liver, lung, brain	GCDFP+, mammoglobin+, GATA3+, CK7+, ER+
Prostate	Bone, lymph node	PSA+, PAP+, NKX3-1+
Gastro-duodenal	Liver, lung, bone	CK7+, CK20+, CDX2, TTF1-
Colorectal	Liver, lung	CK20+, CDX2+, MUC2+, STATB2+, CK7-
Pancreatico-billiary	Liver, peritoneum, lung	CK7+, CK20+, MUC1+, CEA+, CA-19.9+, CA-125+, CK17+
HCC	Lung, peritoneum	Hep-Par 1+, Glypican 3+, CK8+, CK18+
Ovarian	Liver, lymph node	Mucinous: CD7+, CD20+, MUC+
		Non-mucinous: CK7+, CA125+, WT1+, PAX8+
RCC	Bone, lung, liver	RCC Markers+, CK+, EMA+, vimentin+, CD10+, PAX 2+, PAX 8, CK7-, CK20-
Melanoma	Skin, subcutaneous tissue	HMB45+, MelanA+
Neuroblastoma	Bone, bone marrow	Synaptophysin+, CD56+, vimentin variable, neurofilament protein variable
ES/PNET	Bone, bone marrow	Synaphtophysin+, CD56+, FLI1+, CD99+
Head & neck carcinoma	Loco-regional spread, distant metastasis very rare	Squamous cell carcinoma: CK cocktail+, p63+
Urothelial carcinoma	Lymph node, bone, lungs	CK7+, CK20+, high molecular weight cytokeratin, Urothelin, p63

CNS, central nervous system; SCC, squamous cell carcinoma; CK, cytokeratin; TTF, Thyroid transcription factor; ER, estrogen receptor; CEA, carcinoembryonic antigen; PSA, prostate specific antigen; MUC, mucin; CA, carbohydrate antigen; HCC, hepatocellular carcinoma; PAX, paired-box gene; EMA, epithelial membrane antigen; RCC, renal cell carcinoma; ES/PNET, ewing's sarcoma/primitive neuroectodermal tumor.

to establish the definite primary origin of the metastatic disease (**Table 1**).

Approach for IHC markers

Step 1: Determine cell line of differentiation using markers like Cytokeratin (CK), vimentin, LCA (CD45) and S-100. If morphology is suggestive of certain line of differentiation then this step can be skipped.

Step 2: If carcinoma or related, then determine the subtype, whether adenocarcinoma (CK7, CK20, CDX2, PSA etc), squamous cells carcinoma (p63) or neuroendocrine carcinoma (synaptophysin, chromogranin, CD56), mesothelioma (WT1, calretinin, D2-40) or germ cell tumor (AFP, HCG, PLAP).

Step 3: If adenocarcinoma, then following sites are considered and immunohistochemical panel is decided accordingly; lung, small intestine, stomach, colon, pancreatobiliary tract, ovary, breast and prostate (**Table 1**).

Step 4: Cell specific markers are used to identify certain types of primary sites of origin like breast (GCDFP, Mammoglobin), prostate (PSA), Lung (TTF-1) and Liver (Hep Par-1, glypican 3).

Step 5: Exhaustive radiological (e.g. PET/CT scan) and serum tumor markers (e.g. CEA, ALP, CA125 etc), to confirm the site of origin and clinical correlation with histological diagnosis.

New techniques and future research

There is a clear need to improve the sensitivity and specificity of current diagnostic scans and develop label-free technologies that together can provide a whole-body picture of micrometastatic burden to define prognosis. Phenotypic heterogeneity of solid cancers has been investigated at the primary site at single-cell resolution and recently single cell RNA-sequencing has been employed to identify the cell of origin [34]. Recently, high sensitivity disease detecting methods including tumor cell isolation and cell free tumor DNA detection by next generation sequencing (NGS) test have increased the incidence of minimal morphologically occult peripheral blood or bone marrow detection of metastatic neoplasms. Mapping the metastatic cancer cascade onto ctDNA using genetic and epigenetic clonal tracking is a powerful tool to practice precision medicine and target therapy. Currently, methods for automatic differential counting of peripheral blood are readily available commercially. However, morphological assessment and differential counting of BM smears are still performed manually. This procedure is tedious, time-consuming and laden with high inter-operator variation. Recent studies suggested that in patients with clinical

history of cancer, the Artificial Intelligence (AI)-Based Morphogo system may be a useful screening tool in the identification of metastatic cancer cells in the bone marrow aspirate smears [11]. Despite advances in cancer detection and therapy, residual disseminated disease remains present but undetected in a considerable proportion of patients whose primary tumor has been successfully treated. This residual disease can be present as micrometastases, defined as multicellular secondary tumor cell clusters, or as disseminated single tumor cells (DTCs) that are currently too small to detect in clinical diagnostic scans and persist as potential sources of subsequent metastatic relapse. As the morphological and biochemical characteristics of tissue sites primed for metastatic colonisation become more clearly defined, it was proposed that the capacity of AI to detect overt metastasis will soon transfer to the ability to detect these pre-metastatic niches with equivalent accuracy. Such improvements in current clinical diagnostic pipelines will enable the early detection of metastatic dissemination to accurately prioritize patients for the most effective treatment [35]. Tumours comprise a heterogeneous collection of cells with distinct genetic and phenotypic properties that can differentially promote progression, metastasis and drug resistance. Emerging single-cell technologies provide a new opportunity to profile individual cells within tumours and investigate what roles they play in these processes. Single-cell RNA-seq (scRNA-seq) methods enable high-dimensional analyses of cells at the transcriptomic level, highly multiplexed imaging methods provide an image of every cell and thereby allow subcellular localization of proteins as well as morphological assessment [36].

Differential diagnosis

Common differential diagnosis of BM metastasis by non-hematological malignancies are: multiple myeloma and lymphomas, especial in cases with anaplastic morphology, which can be differentiated on BM biopsy with IHC or flowcytometric immunophenotyping by ascertaining the nature of cells infiltrating BM. Lymphomas can be established by initial positivity of LCA with further lineage specific marker positivity. Myeloma also can be established on CD38 and CD138 positivity. Sclerosing bone conditions as intramedullary osteosclerosis or osteopetrosis can potentially mimic sclerotic metastatic disease to bone. Sometimes, dye residues artifact, hematopoietic islands, megakaryocyte clumps, crushed or degenerated cells, osteoblastic and osteoclastic cells and stromal macrophages can be confused with non-hematopoietic originated malignant cells [37].

To conclude, non-hematopoietic malignancies with BM metastasis is rare and associated with poor prognosis. Diagnosis is critical for tumor staging, treatment selection and prognostic risk stratification. It is also helpful in ongoing monitoring residual disease after treatment and predicting relapse. Patients with BM metastasis may have initial presentation similar to hematologic malignancy especially when the primary tumor is not evident. The bone marrow biopsy is a useful tool for identifying the metastatic deposits and along with immunohistochemistry it not only confirms the extent of involvement but also detect its origin. Bone marrow examination has another advantage in cases of carcinoma of unknown origin where the primary site may not detected by radiological study, however the histo-morphological study helps in assessment of the primary site of solid malignancy. IHC can further helpful in identification of dual malignancies infiltration to BM. Thus a thorough knowledge of morphology with IHC is essential for correct diagnosis and early management.

Disclosure of conflict of interest

None.

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