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Pathology

PROGNOSTIC INFORMATION FROM CHEMOTHERAPY-ASSOCIATED CHANGES IN BONE MARROW METASTASIS OF NEUROBLASTOMA- A CASE REPORT

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ABSTRACT Neuroblastoma is the most common extra-cranial solid tumour in children. Bone marrow examination is a part of diagnosis and staging workup of neuroblastoma. Chemotherapy forms mainstay of treatment and post-chemotherapy histological changes are an important indicator of prognosis. A 4-year-old male child came with complaints of fever and hepatomegaly. Investigations revealed a retroperitoneal neuroblastoma. Bone marrow was involved by neuroblasts (stage 4S). Platinum-based chemotherapy was started and post-induction phase bone marrow showed differentiation of neuroblasts to ganglion cells and schwannian stroma (tumour load <5%, minimal disease). The patient was lost to follow up thereafter. The patient returned after one year with the reappearance of neuroblasts (tumour load >20%, relapse) in the bone marrow. Use of immunohistochemical markers like chromogranin and S100 are helpful to map the tumour load and identify the tumour cells when they are sparse. Persistence / reappearance of neuroblasts post-chemotherapy or increasing tumour load indicate a relapse.

KEYWORDS : Neuroblastoma, Chromogranin, S100, Ganglion cells, Schwann cells, Cisplatin

INTRODUCTION:

Neuroblastoma is a neural crest-derived malignancy presenting the early childhood with >40% cases presenting in children <1 year. (1) Bone marrow involvement in neuroblastoma signifies an advanced disease and the stage of the tumour is stage 4 or 4S when bone marrow is involved according to the International Neuroblastoma Staging system. (2) The presence of the metastatic disease is a powerful predictor of a poor outcome and he Internationtal Neuroblastoma Risk Group uses it for risk categorisation and selection of appropriate therapy for the patient. (3)(4)

The diagnosis of neuroblastoma requires histopathological examination of the primary or the metastatic sites. Histopathology of neuroblastoma can range from undifferentiated small round blue cells to a well-differentiated form consisting of ganglion cells and schwannian stroma. The histopathology of the primary site can be different from the metastasis and either can show a differentiated form while the other is undifferentiated. (3) (5) For staging of neuroblastoma, a bilateral bone marrow examination including aspirate smears and biopsy is recommended. (6) Use of immunohistochemistry with neuroendocrine markers like CD56, chromogranin and synaptophysin has increased in recent times for accurate detection and quantification of tumour load in metastatic disease. Also, utilization of stains like S100 is useful to identify a neural/schwannian differentiation in the tumour. (7)

The most common post-chemotherapy change observed in the metastasis is a variable degree of differentiation in the neuroblasts to ganglion cells and presence of a schwannian stroma. An increase in fibrosis and bone marrow necrosis is seen following repeated cycles of chemotherapy. The presence of undifferentiated neuroblasts after induction phase is a poor prognostic factor. (8)(9) A revision to the International Neuroblastoma Response Criterion in 2017 outline the morphological changes in bone marrow metastasis to determining response to therapy.

Here we report a case with emphasis on spatial changes post-chemotherapy in a patient of neuroblastoma and its prognostic implication.

CASE HISTORY:

A 4-year-old male child presented with complaints of fever and pain abdomen. Examination revealed hepatomegaly and left flank tenderness. Investigations showed severe anaemia (6.6 g/dl) and raised lactate dehydrogenase (513 IU/L). Contrast enhanced computerised tomography of abdomen showed a left infra-renal lesion with calcific foci and skeletal metastasis. The 24-hour Homovanillic acid (HVA) levels were elevated (72.9 mg/ g creatinine) and metaiodobenzylguanidine (MIBG) scan was corroborating of metastatic neuroblastoma. Bone marrow aspirate and biopsy at diagnosis showed infiltration by small round blue cells positive for Chromogranin and negative for LCA and cytokeratin (aspirate and biopsy >50%), suggestive of a poorly differentiated neuroblastoma. [Figure 1]

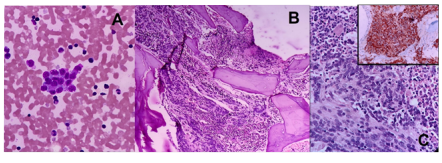

Figure 1: A- Bone marrow aspirate smear showing clusters of small round blue cells (Leishman, x200). B,C - bone marrow biopsy showing infiltration by sheets of small round blue cells (B- H&E,x100; C- H&E, x400) positive for chromogranin(20x) (inset C).

The patient was started on CCG 3891 protocol along with appropriate transfusion support. Post 5 cycles of chemotherapy, the patient developed neutropenia following which G-CSF was administered. Bone marrow reassessment showed aspirate smears with marked granulocytic hyperplasia, predominant immature forms and was attributed to the G-CSF.

Bone marrow biopsy sections revealed differentiation of neuroblasts to ganglion cells and abundant schwannian stroma (ganglioneuroma like differentiation), the undifferentiated neuroblasts were not noted in the sections. The total tumour load in the biopsy sections was <5%, suggestive of minimal disease status. [Figure 2]

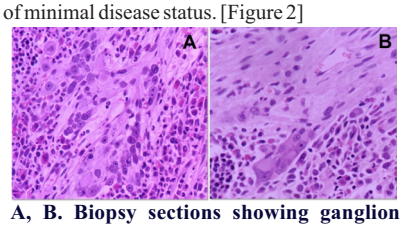

Figure 2: A, B. Biopsy sections showing ganglion cells and Schwann cell differentiation (H&E,x400).

The patient was lost to follow up post 6 cycles of chemotherapy and did not undertake the consolidation phase of the therapy. The patient returned after 1 year with pancytopenia and a leucoerythroblastic blood picture. The bone marrow aspirate was a dry tap but showed clusters of small round blue cells. Bone marrow biopsy sections revealed extensive Schwann cell differentiation and few interspersed ganglion cells. The neuroblasts were seen in small interstitial clusters. The tumour burden in the biopsy sections was > 20% suggestive of a relapse/ progressive disease. [Figure 3 and 4]

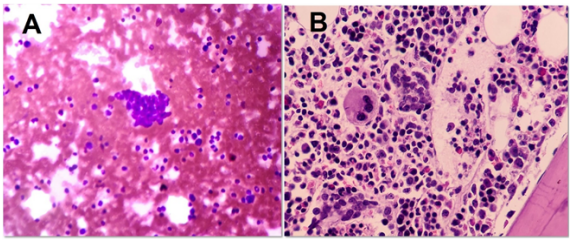

Figure 3: A- Aspirate showing clusters of small round cells (Leishman, x100). B- Biopsy sections showing interstitial clusters of neuroblasts (H&E, x200).

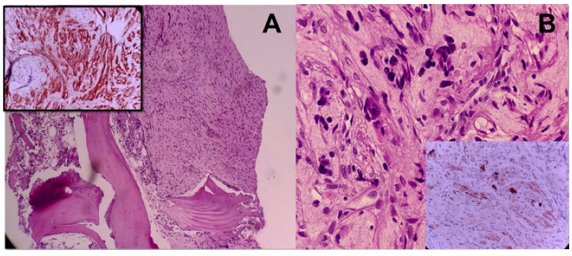

Figure 4: A- Biopsy showing Schwann cell differentiation (H&E, x100). Positive for S100 (inset). B- Biopsy showing scattered ganglion cells (H&E, x200), positive for Chromogranin (inset)

DISCUSSION:

Bone marrow assessment of children with neuroblastoma holds importance in determining the tumour load and in risk stratification of the disease. According to the International Neuroblastoma Staging System (INSS) involvement of bone marrow by neuroblastoma classifies the patient in stage 4S. International neuroblastoma risk group classifies the patients into risk groups based on the INSS stage, where the patients in INSS grade 1 and 2A classified as low risk, grade 2B, 3 into intermediate risk and stage 4, 4S as high-risk groups. The rationale behind such risk stratification is the response to therapy and prognosis in the risk groups. Implementation of an intensive cisplatin-based regimen with bone marrow harvest for transplantation later has shown to be beneficial in high-risk patients. – The present case was staged as 4S and the patient was managed with cisplatin-based chemotherapy.

Morphology of bone marrow metastasis of neuroblastoma is an important factor in determining the effect of chemotherapy and hence to minimise the interobserver variability, International neuroblastoma response criterion bone marrow working group proposed recommendations for standardising the bone marrow disease assessment and reporting. These recommendations included the sample collection, sample quality, assessment and reporting. Table 1 compiles the salient features of the recommended reporting system.

Table 1: Recommendations for bone marrow assessment and reporting in children with neuroblastoma by Internation neuroblastoma response criterion working group.

	Bone marrow aspirate	Bone marrow biopsy	Immunohistochemistry on trephine biopsy
Sample	Bilateral iliac crest	Bilateral iliac crest	Bilateral iliac crest
Sample quality	Tumour cells>5% no criterion needed. Tumour cells <5%, cytological criteria* recommended.	Review recommended if tumour cells <5% in 1 cm red bone marrow parenchyma	Review recommended if tumour cells <5% in 1 cm red bone marrow parenchyma
Assessment	Low(X60-X100) and high (X600-X1000) magnification assessment for tumour nests (at least 3 tumour cells)	Percentage of the tumour, hematopoietic tissue and length (mm) of marrow segment examined	At least two antibodies in three sections
Reporting	Categories, 0%, <5%, 5-<20%, 20- <50%, and >50%	Percent of tumour infiltration in bone marrow space and length (mm) of marrow segment evaluated	Percent of tumour infiltration in bone marrow space and length (mm) of marrow segment evaluated

*cytomorphological inclusion criterion: small round blue nuclei larger than lymphocytes with granular chromatin and cytoplasmic rim less than half of nuclear diameter.

Keeping up with the reporting criterion the tumour load in the present case was assessed to be >50% at diagnosis. Following five cycles of chemotherapy the aspirate did not show any infiltration however the biopsy showed <5% infiltration. After a year of being lost to follow up the aspirate showed 5% -<20% tumour infiltration while biopsy revealed a tumour load of 20%- <50%.

The recommendations also defined the role of histomorphology in determining the response to chemotherapy. A complete response (CR) was defined as the absence of tumour on a reassessment of marrow, progressive disease (PD) was associated with >5% tumour infiltration or doubling of tumour infiltration in marrow on reassessment. A minimal disease (MD) was defined as 0-5% tumour infiltration on reassessment while presence of >5% tumour infiltration but not meeting criterion for CR, PD or MD was labelled as stable disease (SD). (4) The present case showed an MD status following five chemotherapy cycles and a PD/ Relapse when the patient returned after being lost to follow-up for a year.

The presence of tumour infiltration after the chemotherapy has poor prognostic implication. (12) Presence of differentiating neuroblasts to ganglion cells at diagnosis does not affect the prognosis however documentation of differentiation in the reassessment phase points to a better prognosis and persistence of undifferentiated neuroblasts a poor prognosis. (4)(10)(11)

In conclusion metastatic neuroblastoma deposits in bone marrow initially consist of undifferentiated or differentiating neuroblasts regardless of the primary tumour subtype. The neuroblasts show differentiation after chemotherapy, and a tumour load in a satisfactory biopsy helps to reflect upon the response to therapy. Newly appearing poorly differentiated neuroblasts after treatment might be an indicator for poor prognosis making a thorough examination of bone marrow smears imperative for better patient care.

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