CORRESPONDENCE

Bone Marrow Buffy Coat Cell Block for Confirmation of Isolated Bone Marrow Relapse in Medulloblastoma

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Dear sir,

A 12 year old male, a follow up case of Medulloblastoma diagnosed in January 2013 presented in September 2014 with fever off and on since 1 month. He also complained of associated body ache and pain in right leg. Magnetic resonance imaging (MRI) pelvis revealed increased intensity in ilial bones, both femurs and lower lumbar vertebrae suggesting of infiltrative bone disease. His complete blood counts showed a hemoglobin of 8.5 g/dL, total leucocyte count of 4700/ μ L and a platelet count of 3,66,000/ μ L. The differential count was: Neutrophils 68%, lymphocytes 28%, monocytes 2%, eosinophils 1% and metamyelocytes 1%. There was only ~1 nucleated red blood cell per 100 white blood cells. The patient underwent bone marrow aspiration (BMA) and biopsy (BMBx) to rule out bone marrow relapse. The bone marrow smears showed extensive necrosis with only a few intact clusters of small round cells. Normal marrow components including erythroid cells, myeloid cells and megakaryocytes were

completely absent. The bone marrow biopsy showed extensive areas of necrosis (Fig. 1) and residual spaces were fibrotic. Immunohistochemistry (IHC) for Synaptophysin was noncontributory.

To exclude a second malignancy and prove bone marrow relapse, repeat bone marrow was done. A bone marrow sample was also collected in EDTA so that in case of a suspicion of leukemia, flow cytometric immunophenotyping could be performed. Repeat BMA showed only a few clusters of malignant small round cells with presence of necrosis. The differentials were: relapsed medulloblastoma or Ewing's sarcoma. Considering the problems faced earlier with IHC on a necrotic and fibrotic BMBx, we attempted to collect cells from BMA sample in EDTA. A buffy coat was prepared from this sample by putting it in Wintrobe's tube and spinning it at 2000 g for 10 min. The supernatant plasma was discarded and the buffy coat so collected was isolated in another plastic tube. Since it is important to hold these cells, 250 μ L citrated normal plasma was added to the buffy coat and a clot was generated using 250 μ L calcium chloride. The clot was separated

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Fig. 1 Hematoxylin and eosin stained bone marrow biopsy showing necrotic tumour cells ($\times 200$)

Fig. 2 IHC on cell-block. **a** Clusters of small round cells positive for Synaptophysin (×400). **b** Small round cells negative for CD99 (×200)

Fig. 3 **a** Hematoxylin and eosin stained bone marrow biopsy section with malignant small round cell clusters in a fibrotic background (×200). **b** Tumour cells staining positive for Synaptophysin (×200). **c** CD99 negative malignant cells (×200)

after 30 min and was added to formalin. This cell block was then processed as per routine histopathology processing protocol. The cell block sections revealed the presence of dense aggregates of small round cells. These cells had a high N:C ratio, scant pale basophilic cytoplasm, round nuclei, coarse chromatin with a few showing nucleoli. We asked for Synaptophysin and CD99 immunocytochemistry based on our earlier differentials. The ICC on the cell block was positive for Synaptophysin while CD99 was negative (Fig. 2). The BMBx section also showed few clusters of malignant small round cells in a fibrotic and necrotic background. IHC on the BMBx was also conducted to confirm the findings as well as to compare the staining quality. The results of IHC on the BMBx were similar to ICC on the cell block but the cell block gave sharper positive reactions as there was no fibrosis (Fig. 3).

Bone marrow metastases are commonly encountered in pediatric small round cell tumours like Neuroblastoma [1], Ewing's sarcoma [2] and embryonal rhabdomyosarcoma [3]. Among the central nervous system, Medulloblastoma has the highest risk for bone marrow metastases [4]. Metastases from these pediatric tumours may resemble acute leukemia [5]. Although flowcytometric immunophenotyping is essential to diagnose acute leukemia, there is a lack of helpful markers in cases of metastasis. There are reports depicting the use of CD45, CD56, CD57 and CD90 for identification of Rhabdomyosarcoma cells [3]. To demonstrate the presence of

infiltrating neuroblastoma cells, a panel of CD56, CD45 and NB 84 has been used [3]. These markers are not readily available and are not routinely applied for diagnosis of metastases.

To confirm bone marrow metastases in the present case, we combined the cell block technique with malignant cell concentration using the buffy coat technique. Buffy coat smears are usually prepared from peripheral blood and are useful to evaluate for parasites such as microfilaria and malaria, to concentrate blasts, megakaryocytes, hairy cells, demonstrate LE cells and neoplastic cells in solid tumours. We used the same technique to concentrate malignant cells from BMA and after generating a clot from this EDTA bone marrow buffy coat used the cell block technique to obtain tissue for applying immunocytochemistry. Our aim is to highlight the utility of bone marrow buffy coat cell block in diagnosing metastatic tumors and distinguishing them from primary bone marrow malignancies.

Compliance with Ethical Standards

Conflict of interest None.

Ethical Approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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