

BRIEF REPORT

Vacuolated Neuroblastoma Cells Mimicking FAB L₃ Lymphoblasts in Bone Marrow Aspirates

Patricia Alcasabas, MD,* Andrew Dettore, DO, Bülent Özgönenel, MD, Kanta Bhambhani, MD, Yaddanapudi Ravindranath, MD, and Süreyya Savaşan, MD

Abundant cytoplasmic vacuolation of neuroblasts has been noted on bone marrow aspirate (BMA) smears of two patients with metastatic neuroblastoma. Occasional tumor cells were dispersed as individual cells as well as in clumps. These cells had basophilic cytoplasm and several nucleoli, reminiscent of L₃ lymphoblast morphology. Flow cytometric analysis of the bone marrow mono-

nuclear cells and neuron-specific enolase staining of the bone marrow biopsy samples further distinguished the cells as neuroblasts. Cytoplasmic vacuolations of neuroblasts may be a feature of metastatic neuroblastoma cells in BMA smears. *Pediatr Blood Cancer* © 2006 Wiley-Liss, Inc.

Key words: acute lymphoblastic leukemia; bone marrow aspiration; cytoplasmic vacuolation; L3 type; neuroblastoma

INTRODUCTION

Neuroblastoma is classically composed of small round blue cells forming pseudorosettes around fibrillary stroma. Prominent cytoplasmic vacuoles are a hallmark of the L₃ subtype of acute lymphoblastic leukemia (ALL) in the French-American-British classification. Cytoplasmic vacuolation has also been observed in medulloblastoma [1], Ewing sarcoma, and rhabdomyosarcoma [2,3], but rarely described as a morphologic feature in primary neuroblastoma tumor tissue biopsies [4,5]. Hence, vacuolated metastatic neuroblastoma morphologically can mimic acute leukemia in bone marrow aspirate (BMA) smears, especially in cases where the bone marrow is replaced by non-cohesive tumor cells [6,7]. Here we describe two cases of metastatic neuroblastoma with significant cytoplasmic vacuolations of the neuroblasts.

CASE PRESENTATIONS

Case 1

The first patient was a 2-year-old male, who initially presented at 19 months of age with a large mass originating from the right adrenal gland. Histological examination of the mass revealed stroma-poor neuroblastoma with a high mitosis-karyorrhexis index (MKI) and amplification of the *MYCN* gene. The bone marrow was not involved with neuroblastoma at that time. More than 90% reduction of the tumor was achieved after five courses of chemotherapy with combinations of cisplatin, carboplatin, etoposide, doxorubicin, and cyclophosphamide. Treatment was discontinued because the parents did not consent for further chemotherapy.

Eight months after the last course of chemotherapy, the patient sustained a pathologic fracture of the right tibia. Imaging studies revealed tumor recurrence with extensive osseous metastases. BMA smears stained with Wright-

Giemsa showed either isolated or rare clumps of tumor cells with abundant, cytoplasmic vacuolation. They displayed basophilic cytoplasmic staining and had several nucleoli reminiscent of L₃ lymphoblast morphology (Fig. 1A). Flow cytometric analysis of the bone marrow mononuclear cells on an Epics XL machine (Coulter, Miami, FL) revealed the presence of cells which were negative for CD45, CD19, and surface immunoglobulin, but positive for CD9 and CD56, consistent with a neuroectodermal origin (Fig. 2) [8]. Trephine biopsy of the bone marrow showed aggregates of small blue cells with positive neuron-specific enolase staining. The patient received several courses of chemotherapy including cyclophosphamide and topotecan, as well as surgical excision of the primary adrenal mass. He died 7 months after his relapse.

Case 2

The second patient was a 3-year-old female, who presented with right lower extremity pain, limping, periorbital ecchymoses, and anemia. Imaging studies showed a right-sided adrenal mass and widespread osseous metastases. Clumps of small blue cells with abundant cytoplasmic vacuolation were seen in bone marrow aspiration smears (Fig. 1B). Trephine biopsy of the bone marrow showed sheets of malignant small round blue cells with scanty cytoplasm, inconspicuous nucleoli, and a neurofibrillary background with positive neuron-specific enolase staining. *MYCN* amplification and trisomy 8 were detected in the metastatic

Division of Hematology/Oncology, Children's Hospital of Michigan, Wayne State University, Detroit, MI

*Correspondence to: Patricia Alcasabas, Division of Hematology/Oncology, Children's Hospital of Michigan, 3901 Beaubien Boulevard, Detroit, MI 48201. E-mail: tricia_alcas@yahoo.com

Received 28 April 2005; Accepted 16 November 2005

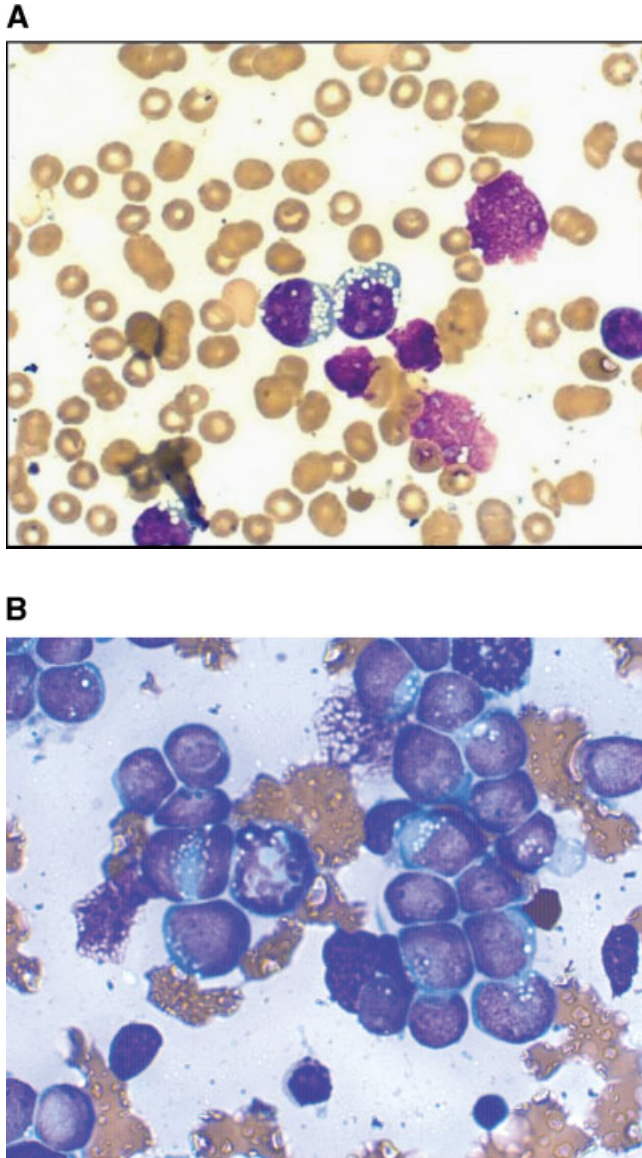


Fig. 1. Neuroblasts with prominent cytoplasmic vacuolation are shown on bone marrow aspiration smears of the first (A) and second (B) case (Wright-Giemsa, 100 \times). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

bone marrow neuroblasts using fluorescence in situ hybridization technique.

DISCUSSION

Metastatic neuroblastoma of the bone marrow typically displays large clumps of tumor cells that can be easily detected with the examination of BMA smears using low-magnification [7]. The presence of Homer-Wright rosettes and a blue-gray, amorphous, and fibrillar background material more specifically point to a diagnosis of metastatic neuroblastoma on review of tissue biopsy specimens [9].

The examination of the bone marrow is one of the primary tests for patients presenting with anemia and bone pain. The absence of an abdominal mass on either physical examination or imaging studies could lead the diagnosis toward leukemia. However, there are rare cases of neuroblastoma presenting without an identifiable primary site but with bone marrow metastases. In such patients, the presence of cytoplasmic vacuolations may potentially make the diagnosis of neuroblastoma appear unlikely. There have been anecdotal cases of neuroblastoma initially diagnosed as acute leukemia [10,11]. Secondary acute myeloid leukemia and co-occurrence of leukemia with neuroblastoma have also been reported [12–15].

Glycogen and lipid accumulation comprise the cytoplasmic vacuoles in Ewing sarcoma and Burkitt lymphoma, respectively [4]. In neuroblasts under in vitro conditions, cytoplasmic vacuoles have been observed in degenerating neuroblastoma cell lines and following exposure to prostaglandins [16] or iron [17,18]. Although our first case had documented transfusional iron overload at the time of BMA, the second patient did not. It is very premature to draw a conclusion that the presence of vacuoles might be associated with the iron overload. Hence, the etiology of neuroblast vacuolation in these two cases remains unknown. We have also reviewed the stained BMA smears of the eight other cases with metastatic neuroblastoma followed at our hospital in the last 4 years. None of these had neuroblasts with cytoplasmic vacuolations.

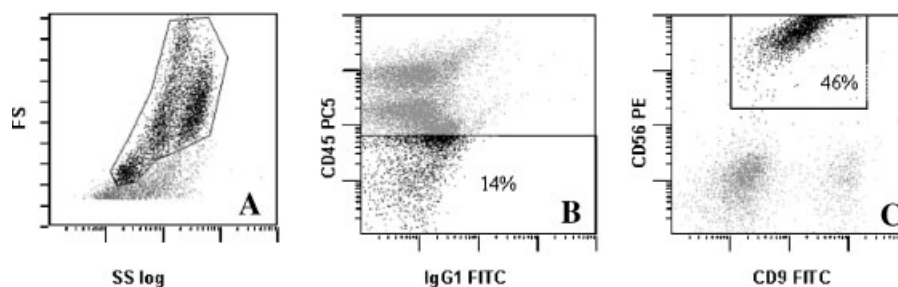


Fig. 2. Whole bone marrow was stained with CD45-PC5, CD9-FITC, and CD56-PE (Immunotech, Brea, CA). Control isotypes used were IgG1-FITC and IgG2A-PE (Immunotech, Brea, CA). Nucleated cells were gated using forward scatter (FS) and side scatter (SS) characteristics to exclude platelets, debris, and erythrocytes. (A) CD45-negative to dim events (B) were sent to two parameter histogram of CD9-FITC versus CD56-PE and double-positive events were enumerated (C). CD45-negative, CD9-positive, and CD-56-positive cells represent neuroblasts.

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