

Can flow-cytometry be used at diagnosis and follow-up in neuroblastoma with bone marrow involvement?

YAVUZ KÖKSAL¹, ISMAIL REİSLİ¹, FİGEN DOĞU², CANAN UÇAR¹, HASAN ACAR³, ÜMRAN ÇALIŞKAN¹

¹Selçuk University, Meram Medical Faculty, Department of Pediatrics, Konya, ²Ankara University, Faculty of Medicine, Department of Pediatric Immunology, Ankara, ³Selçuk University, Meram Medical Faculty, Department of Genetics, Konya-Turkey

ABSTRACT

A 4.5-year-old boy was admitted to our clinic with fever, which had persisted for one month, weakness, and hand, arm and knee pain. Physical examination was remarkable for pallor, the movement of the right knee was painful and restricted, yet both knees were same in diameter. The peripheral blood smear revealed moderate immature lymphocytosis. Bone marrow aspiration revealed uniform cells with narrow cytoplasm and hyperchromatic nuclei which formed a "rosette" scene. On bone marrow flow-cytometry examination, CD45(-)/CD44(+)/CD56(+) cells were 70% positive. The urinary VMA level was 41.6 mg/24 h. There was an anterior paramediastinal mass and vertebral destructions in thoracal computed tomography (CT). Cranial CT and magnetic resonance imaging (MRI) revealed duramater involvement and numerous subdural tumors. A paravertebral mass that extended into the spinal canal was determined with spinal MRI. Stage IV neuroblastoma (NB) was diagnosed and ENSG-3C chemotherapy protocols including cisplatin, etoposide, ifosfamide, mesna, vincristine and adriamycine was given. After two cycle of the chemotherapy, bone marrow aspiration was normal and CD45(-)/CD44(+)/CD56(+) cells were 5% positive. Following treatment, he has been in remission for 18 months. Flow-cytometry may be useful for the diagnosis and follow-up of NB with bone marrow involvement. [Turk J Cancer 2006;36(1):27-30].

KEY WORDS:

Neuroblastoma, flow-cytometry, anterior mediastinum, duramater, children

INTRODUCTION

Neuroblastoma, which originates from the sympathetic chain, is the most common extracranial solid tumor in childhood. The tumor is mainly composed of neuroblasts with occasional ganglion cells. A pathologic diagnosis is made from tumor tissue obtained by biopsy. However, NB can be diagnosed without a tumor biopsy if the patient has neuroblasts in bone marrow and elevated VMA or HVA in the urine (1). There do exist some studies concerning the ability to recognize neuroblasts using flow-cytometry as well as identifying its clinical characteristics (2,3). We report a case in which a child was diagnosed with advanced NB by examination of bone marrow aspiration, flow-cytometry and high urine VMA level.

CASE REPORT

A 4.5-year-old boy was admitted to our clinic with fever which had persisted for one month, weakness, and hand,

arm, and knee pain. There was no history of recent infection. Physical examination was remarkable for pallor, the movement of the right knee was painful and restricted, yet both knees were the same in diameter. The rest of the physical examination was normal. The peripheral blood count showed a hemoglobin level of 7.8 gr/dL, a platelet count of 318000/mm³, and white blood cell count of 6300/mm³. The peripheral blood smear revealed moderate immature lymphocytosis. The erythrocyte sedimentation rate was 105 mm/h. Bone marrow aspiration revealed uniform cells with narrow cytoplasm and hyperchromatic nuclei, which formed “rosette” scene (Figure 1). On bone marrow flow-cytometry examination, CD45(-)/CD44(+)/CD56(+) cells were 70% positive (Figure 2A and B). The serum chemistries were normal except for a highly elevated lactate dehydrogenase level. The ferritin level was normal and urinary VMA level was 41.6 mg/24 h (1-11 mg/24 h). There was an anterior paramediastinal mass and vertebral destructions in thoracic CT (Figure 3). Cranial CT and MRI revealed duramater involvement and numerous subdural tumors. A paravertebral mass that extended into the spinal canal was observed with spinal MRI and abdominal CT. ¹³¹I- Metaiodobenzylguanidine scintigraphy (MIBG) was negative. With these findings, stage IV NB was diagnosed. The ENSG-3C chemotherapy protocol including cisplatin, etoposide, ifosfamide, mesna, vincristine and adriamycin was given (4). After second cycle of the chemotherapy, bone marrow aspiration was normal and CD45(-)/CD44(+)/CD56(+) cells were found 5% positive during controls. There were marked regressions in the anterior paramediastinal, dural, subdural and paravertebral masses. In the follow-up, he has been in remission for 18 months.

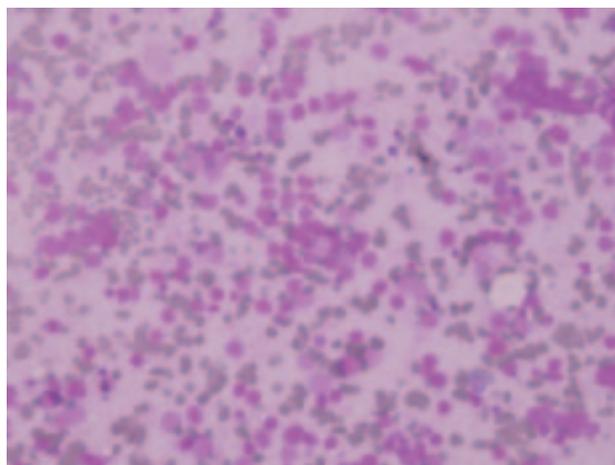


Fig 1. Uniform cells with narrow cytoplasm and hyperchromatic nuclei forming “rosette” scene in bone marrow aspiration

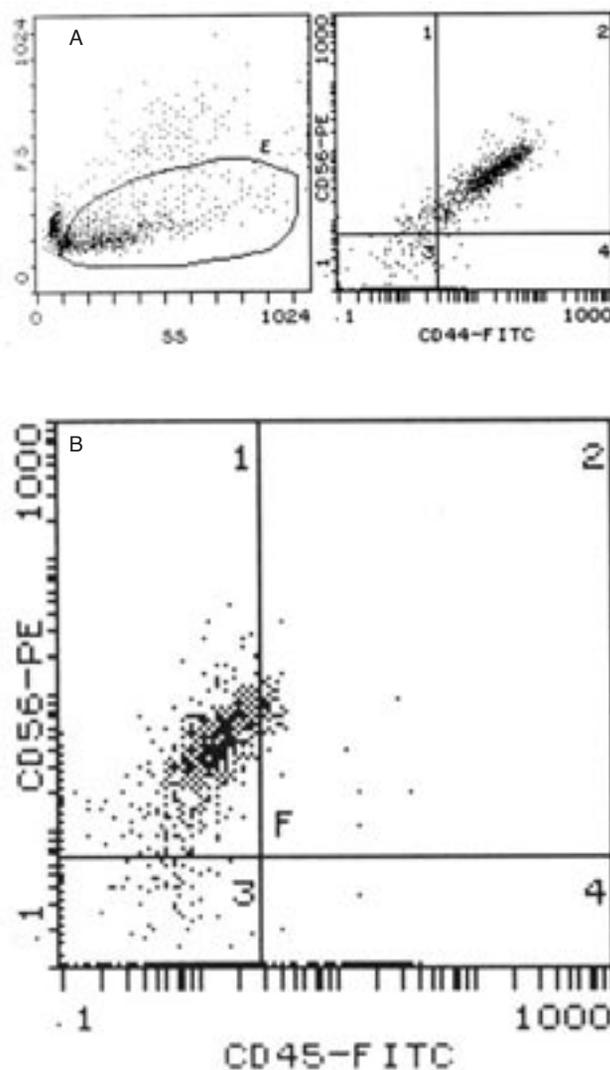


Fig 2 (A,B). CD45(-)/CD44(+)/CD56(+) cells were 70% positive in flow-cytometry

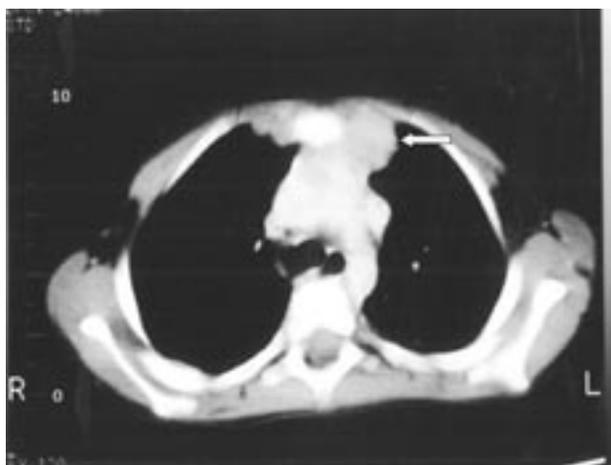


Fig 3. Thoracic computed tomography shows anterior mediastinum involvement

DISCUSSION

Neuroblastomas are the most common extracranial solid tumors seen in children. Metastatic disease is found in more than 50% of children at diagnosis. Childhood NBs usually originate in the abdomen (adrenal glands and extra-adrenal sites), thoracic structures (especially posterior mediastinum) and sympathetic chain. Moreover, bone, bone marrow, liver, cutaneous tissue, as well as pulmonary and brain parenchyma can also be involved as a result of hematogenous spread (1-3).

Anterior mediastinum and duramater involvement is extremely rare. NBs localized in anterior mediastinum in adult patients have been reported (5-10). A NB of the thymus in an adult patient, accompanied by syndrome of inappropriate secretion of ADH has also been documented (9). Brain metastases from extracranial neuroblastoma are rare. They are generally produced by direct extension from metastatic lesions of the skull or dura. The incidence of leptomeningeal or central nervous system parenchymal disease in patients who experience disease recurrence ranges from 1% to 16% (11-13). In the literature, it was reported that out of a total of 14 NB patients with brain involvement, only one of them had duramater disease (14). In our case, with advanced disease at diagnosis, metastatic involvement included the anterior mediastinum, dura mater as well as numerous subdural tumors.

Flow-cytometry is not a routine method used during diagnosis of NB. But in recent years, this method has demonstrated its value in treatment follow-up and for verification of residual mass by confirming NB cells circulating in peripheral blood and bone marrow (2,3). It is known that NB cells express CD45(-)/CD44(+)/CD56(+)/CD81(+)/CD9(+) antigens (2,3,15,16). It is an effective method to rapidly detect NB cells in bone marrow (16). In our case, analysis of the bone marrow sample with flow-cytometry revealed expression of CD45(-)/CD44(+)/CD56(+) in NB cells. After second cycle of the chemotherapy, CD45(-)/CD44(+)/CD56(+) positivity was 5%.

Prognostic factors of NB are stage, age, N-myc, high VMA level, histology (1). However, CD44 expression in flow-cytometry is also a good prognostic criterion, independent from the stage, age and N-myc amplification (17,18). Our patient had poor prognostic factors such as age, stage and elevated urinary VMA. However, response to the treatment was good. We think that our patient's response to the therapy is related to CD44 expression.

In conclusion, NB cases involving the anterior mediastinum, duramater and brain involvements are extremely rare. Despite the presence of advanced disease, we think that CD44 expression is an indicator of good prognosis. The flow-cytometry may be helpful for diagnosis and follow-up of NB with bone marrow involvement.

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