# Acta Medica Okayama

Volume 37, Issue 5

1983 October 1983

Article 10

# Detection of metastatic neuroblastoma cells in bone marrow aspirates by formaldehyde induced fluorescence histochemistry.

Kiminao Mizukawa\* Ryuuhei Fujii<sup>†</sup> Tatsuro Fujisawa<sup>‡</sup> Nagayasu Otsuka<sup>\*\*</sup> Shinsaku Imashuku<sup>††</sup>

\*Okayama University, <sup>†</sup>Okayama University, <sup>‡</sup>Okayama University, \*\*Okayama University,

<sup>††</sup>Kyoto Prefectural University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

## Detection of metastatic neuroblastoma cells in bone marrow aspirates by formaldehyde induced fluorescence histochemistry.\*

Kiminao Mizukawa, Ryuuhei Fujii, Tatsuro Fujisawa, Nagayasu Otsuka, and Shinsaku Imashuku

## Abstract

Metastatic neuroblastoma cells in bone marrow aspirates were examined by the formaldehyde induced fluorescence histochemical method. With this method we could easily identify abnormal cells as metastatic neuroblastoma cells by observing catecholamine green colored fluorescence in their cytoplasma. This formaldehyde induced fluorescence histochemical method is significantly useful for the diagnosis of metastatic neuroblastoma of the bone marrow.

**KEYWORDS:** metastatic neuroblastoma cells, bone marrow aspirate, formaldehyde induced fluorescence histochemistry

\*PMID: 6650226 [PubMed - indexed for MEDLINE] Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL Mizukawa et al.: Detection of metastatic neuroblastoma cells in bone marrow

Acta Med. Okayama 37, (5), 451-453, (1983)

### — BRIEF NOTE —

### DETECTION OF METASTATIC NEUROBLASTOMA CELLS IN BONE MARROW ASPIRATES BY FORMALDEHYDE INDUCED FLUORESCENCE HISTOCHEMISTRY

Kiminao Mizukawa, Ryuuhei Fujii, Tatsuro Fujisawa, Nagayasu Otsuka and Shinsaku Imashuku\*

Department of Anatomy, Okayama University Medical School and \* Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto 602, Japan

Received May 23, 1983

Abstract. Metastatic neuroblastoma cells in bone marrow aspirates were examined by the formaldehyde induced fluorescence histochemical method. With this method we could easily identify abnormal cells as metastatic neuroblastoma cells by observing catecholamine green colored fluorescence in their cytoplasma. This formaldehyde induced fluorescence histochemical method is significantly useful for the diagnosis of metastatic neuroblastoma of the bone marrow.

*Key words* : metastatic neuroblastoma cells, bone marrow aspirate, formaldehyde induced fluorescence histochemistry.

Neuroblastoma is one of the most common malignant solid abdominal tumors in children. This neuroblastoma characteristically tends to metastasize widely and rapidly to the bone marrow. It is very difficult to identify the metastatic neuroblastoma cells only by means of histological examination with May-Giemsa staining of the bone marrow aspirate smears. This type of tumor characteristically produces and secretes catecholamines, so in order to identify the metastatic neuroblastoma cells, we applied the formaldehyde induced fluorescence histochemical method which visualizes intracellular catecholamines (1).

Materials and methods. The bone marrow aspirates were taken from the iliac crest and occasionally from the sternum of patients who were highly suspicious of having neuroblastoma. The aspirates were thickly smeared on non-fluorescent glass slides and immediately dried with hot air. The specimens were placed in a closed chamber with paraformaldehyde vapor at 80  $\degree$  for one hour and mounted with Entellan. The specimens were examined by a fluorescence microscope equipped with an HBO high pressure mercury lamp, BV excitation filter, dark field immersion condenser and Y-50 barrier filter.

*Resurts and discussion.* Metastatic neuroblastoma cells in the bone marrow smears were typically observed in clumps. These clumps had a syncytial or rosette-like arrangement with indistinct cell boundaries, and the nuclei of these

1

452

K. MIZUKAWA et al.



Fig. 1. May-Giemsa staining of bone marrow aspirates. (disseminated neuroblastoma) Fig. 2. Fluorescence micrograph of a bone marrow aspirate from a patient with disseminated

neuroblastoma. A large number of catecholamine fluorescence positive cells are observed.

Fig. 3. Fluorescence micrograph of singly scattered metastatic neuroblastoma cells in a bone marrow aspirate. (early stage of neuroblastoma)

### Detection of Neuroblastoma Cells

cells were immature and large. Because of the typical morphologic characteristics of neuroblastoma cells, it is not so difficult to identify abnormal cells as metastatic neuroblastoma cells in the advanced stage of neuroblastoma only by May-Giemsa staining (Fig. 1).

In the very early stage, on the other hand, metastatic neuroblastoma cells are not present in clumps and tend to be scattered singly. Thus, it is very difficult to distinguish neuroblastoma cells from reticulum cell sarcoma, lymphosarcoma, Ewing's sarcoma, rhabdomyosarcoma, Wilm's tumors and leukemia cells only by May-Giemsa staining (2, 3).

With the formaldehyde induced fluorescence histochemical method, it is easy to clearly identify the metastatic neuroblastoma cells in bone marrow aspirates by the green catecholamine fluorescence (Fig. 2). Even single neuroblastoma cells may have green catecholamine fluorescence and thus be easily and clearly identified as metastatic neuroblastoma cells (Fig. 3).

#### REFERENCES

- 1. Falck, B., Hillarp, N. -Å., Thieme, G. and Torp, A.: Fluorescence of catechol amines and related compounds with formaldehyde. J. Histochem. Cytochem. 10, 348-354, 1962.
- 2. Finklestein, J.Z., Ekert, H., Isaacs, H. and Higgins, G.: Bone marrow metastases in children with sold tumors. *Am. J. Dis. Child.* **119**, 49-52, 1970.
- 3. Belasco, J.B., McMillan, C.W.: Occult neuroblastoma : difficulties of diagnosis. Am. J. Dis. Child. 133, 858-860, 1979.

3

453