Acute Myelomonocytic Leukemia with Abnormal Eosinophils: A Case Report with Multi-Modality Diagnostic Work-up

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Acute myeloid leukemia (AML) with recurrent genetic abnormalities often carries a favorable prognosis. AML with inv(16)(p13q22) occurs predominantly in younger patients and usually shows granulocytic and monocytic differentiation with abnormal eosinophils. It is referred to as acute myelomonocytic leukemia with abnormal eosinophils (AMML Eo). We report a case in a 27-year-old man with leukocytosis (10.6x10^3/µL with 34% blasts), thrombocytopenia and splenomegaly. Marrow aspiration showed 47% blasts and 33% eosinophils, of which 19% were morphologically abnormal with both eosinophilic and basophilic cytoplasmic granules. Cytochemically, the blasts were positive for myeloperoxidase while the granules of abnormal eosinophils were positive for naphthol ASD chloroacetate esterase. With flow cytometric immunophenotyping the blasts expressed CD13, CD33, CD117, myeloperoxidase and CD34. Marrow trephine showed 90% cellularity with 40% blasts expressing CD34, CD117, and myeloperoxidase on immunohistochemistry. Chromosomal analysis revealed a karyotype of 46, XY, inv(16)(p13q22). This case illustrates a typical AMML Eo confirmed by a multi-modality diagnostic approach including morphology, cytochemistry, flow cytometry, immunohistochemistry, and conventional cytogenetic study. (Chang Gung Med J 2006;29:532-7)

Key words: acute myeloid leukemia, acute myelomonocytic leukemia, chromosome translocation, eosinophil.
CASE REPORT

In February 2005, a 27-year-old Canadian Caucasian man visited the emergency department due to painful swelling of the right leg for one day and body weight loss of 11.3 kg. He was admitted under the impression of cellulitis. However, laboratory tests showed mild anemia (hemoglobin 13.9 g/dL), mild leukocytosis (WBC 10.6 x 10^3/µL) with 34% blasts and 0% eosinophils, marked thrombocytopenia (76 x 10^3/µL), and elevated C-reactive protein (33.4 mg/L) and lactate dehydrogenase (738 IU/L) levels. Abdominal sonography revealed splenomegaly up to 13.5 cm while chest radiography was unremarkable.

He received a bone marrow aspiration and biopsy. Wright-Giemsa and cytochemical stains including myeloperoxidase (MPO), α-Naphthyl butyrate esterase (BE), naphthol ASD chloroacetate esterase (CLE) and combined esterase stain (BE/CLE) were performed on the marrow aspirate. Flow cytometric immunophenotyping was performed using marrow aspirate, which was centrifuged in Biocoll separation solution (Biochrom KG, Berlin, Germany) and the mononuclear cell layer was then re-suspended at 1 x 10^7 cell/mL before incubation with antibodies at room temperature. The suspension was centrifuged, washed with phosphate buffered saline, and incubated with fluorochrome (phycoerythrin [PE], fluorescein isothiocyanate [FITC] or peridinin-chlorophyll [PerCP]) conjugated monoclonal antibodies and analyzed with a FACSCalibur flow cytometer (Becton Dickinson [BD], San Jose, CA) and CellQuest software. The antibody panels included CD14/CD45 (PE/PerCP), CD3/HLA-DR, CD19/κ, CD19/λ, CD19/CD10, CD20/CD5, CD13/CD34, p-glycoprotein/CD33, CD117/CD33, CD7/CD33, CD13/CD56, CD11b/CD33, and CD11c/CD15 (PE/FITC). To detect cytoplasmic antigens, the cellular suspension was pretreated with FACS permeabilizing solution 2 (BD) before incubation with the conjugated antibodies including CD33/cytoplasmic MPO (cMPO) and cCD22/cytoplasmic terminal deoxynucleotidyl transferase (cTdT) (PE/FITC) (all from BD except for TdT, from Serotec, Oxford, UK). Immunohistochemical stains on the biopsy specimen were performed using anti-CD34 (Serotec Inc., Raleigh, NC, USA), CD117 and MPO (Dako Cytomation, Glostrup, Denmark) with pressure-cooking antigen retrieval in citrate pH 6.0 and appropriate positive controls. Cytogenetic study was done on marrow mononuclear cells harvested directly and after a 24-hour culture. Metaphases were banded by a conventional trypsin-Giemsa banding technique and the karyotype was interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).(7)

The marrow aspirate smears showed 47% blasts without Auer rods, 5% monocytes, and 33% eosinophils and their precursors including morphologically normal (14%) and abnormal (19%) populations. In addition to the normal-appearing eosinophilic granules, the abnormal eosinophilic precursors contained large, basophilic granules (Fig. 1A). Cytochemically, the blasts stained positive for MPO while the large, basophilic granules in the abnormal eosinophils stained positive for CLE (Fig. 1B-D). Flow cytometric immunophenotyping showed that these blasts expressed myeloid antigens CD13, CD33, CD117, and MPO in addition to HLA-DR and CD34 (Fig. 2). A fraction (30%) of these blasts expressed CD11c. They were negative for CD3, CD5, CD7, CD10, CD11b, CD14, CD15, CD19, CD20, cCD22, CD56, p-glycophorin, and TdT. The marrow trephine was hypercellular with 90% cellularity and 40% of the cellular components were small to medium-sized blasts. Immunohistochemically, these blasts expressed CD34, CD117 and MPO (Fig. 3). Chromosomal analysis of 22 metaphases revealed that 18 cells exhibited pericentric inversion of chromosome 16, i.e. inv(16)(p13q22) (Fig. 4); while four cells showed a normal karyotype.

After a complete diagnostic work-up, AMML Eo with inv(16)(p13q22) was diagnosed and this patient returned to Canada for treatment.

DISCUSSION

The overall incidence of acute leukemia is approximately 4/100,000 population per year, with 70% of these cases being AML. AML is a heterogeneous clonal disorder with individual cases exhibiting variations in clinical presentation, cellular morphology, therapeutic response and overall prognosis. In a cytogenetic study of 235 cases of AML in Taiwanese, Tien et al. identified 12 cases with...
Fig. 1 (A) Marrow aspirate smear shows blasts and abnormal eosinophilic precursors containing a mixture of normal eosinophilic granules and large, basophilic granules (Wright-Giemsa stain). (B) The blasts are positive for myeloperoxidase. (C) The marrow monocytic component is highlighted by a naphthyl butyrate esterase stain. (D) In addition to myeloperoxidase-positivity as shown in Figure 1B, the abnormal granules in the eosinophilic precursors are positive for naphthol ASD chloroacetate esterase. (1000x)

Fig. 2 (A) The blasts express myeloid antigens CD13/CD34 and (B) cytoplasmic CD33/myeloperoxidase (Flow cytometric immunophenotyping).
inv(16) and 11 of these were AMML Eo, accounting for 24% (11/45 cases) of AML-M4. They found that the patients with inv(16) had the longest median complete remission rate and median survival time, followed by those with t(15;17) and t(8;21). A more recent cytogenetic study of 111 cases of Taiwanese childhood acute leukemia revealed four AML-M4 cases among 30 cases of AML. Of the three AML-M4 cases with cytogenetic abnormalities, one was AMML Eo with t(7;16)(q21;q22). Follow-up information on this patient was not available.

The most important diagnostic clue in our case was the high percentage of marrow eosinophils, more than half of which were morphologically abnormal. Peripheral blood eosinophilia is occasionally encountered as a reactive process secondary to infection/inflammation (allergy, parasitic infection, or drug reaction) and non-myeloid neoplasms such as acute lymphoblastic leukemia, and peripheral T-cell and Hodgkin lymphomas. Clonal eosinophilic proliferations are neoplastic disorders involving clonal expansion of eosinophils in AMML Eo, myelodysplastic syndrome (MDS), and chronic myeloproliferative disorders (CMPD) including chronic myelogenous leukemia, chronic eosinophilic leukemia, and idiopathic hypereosinophilic syndrome. The marrow blast count of 47% in our case excluded the possibility of idiopathic hypereosinophilic syndrome, MDS, and CMPD. Although excess blasts may be seen in the blastic phase of chronic myelogenous leukemia, there was no Philadelphia chromosome on conventional cytoge-

**Fig. 4** Chromosomal analysis shows inv(16)(p13q22) with pericentric inversion of chromosome 16 (arrow).

**Fig. 3** (A) Bone marrow biopsy shows packed marrow with blasts and eosinophils. (H & E stain, 400x). (B) These blasts express CD34 (immunohistochemical stain, 400x).
The altered expression of gene products from genetic aberrations in AML may contribute to the initiation or progression of leukemogenesis. The inv(16)(p13q22) or t(16;16)(p13;q22) result in the fusion of the CBFβ (core binding factor β subunit) gene at 16q22 to the MYH11 gene at 16p13, which encodes the smooth muscle myosin heavy chain. The resultant chimeric protein blocks hematopoietic differentiation that characterizes AML.(10) Recent clinical and experimental studies strongly support the concept that the CBFβ fusion genes are present in potential leukemic precursor cells but that additional mutations, such as those involving mutations involving RTKs and RAS genes, are necessary for transformation.(11) By conventional cytogenetics, inv(16) is a subtle rearrangement that may be overlooked when metaphase preparations are suboptimal. Under these circumstances, the use of interphase fluorescent in situ hybridization and reverse transcription-polymerase chain reaction may be necessary to document the genetic alternations.(1,3)

This case illustrates a typical case of AMML Eo. The presumptive diagnosis was raised because of the immature and mature eosinophils with abnormal cytoplasmic granules (particularly evident at the promyelocytic and myelocytic stages of differentiation) and was confirmed by a multi-modality diagnostic approach including morphology, cytochemistry, immunohistochemistry, flow cytometry, immunohistochemistry, and conventional cytogenetics.

REFERENCES

急性骨髓性白血病合併異常嗜伊紅性白血球：
以多種檢驗方法診斷的一例病例報告

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具有常見之基因異常的急性骨髓性白血病(AML)通常有較高的完全緩解率以及較佳的預後。在16號染色體有inv(16)(p13q22)之基因轉位的急性骨髓性白血病通常好發於年輕的病人，其骨髄中常可以見到不正常的嗜酸性白血球，分類上被歸在AMML Eo或者稱為M4Eo。我們報告一例27歲之病患，其表現為在週邊血液中有白血球增多(10.6×10^9/μL，包含34%芽細胞)以及血小板減少(76×10^3/μL)之情形。腹部超音波顯示脾有腫瘤大達13.5公分。骨髓穿刺結果發現高度40%的芽細胞以及增多的嗜酸性白血球(佔33%)，其中有19%為不正常嗜酸性白血球，其細胞質中同時含有嗜酸以及嗜酸性顆粒。細胞學化染色包含骨髄過氧化酶(MPO)以及naphthol ASD chloroacetate esterase 分別在芽細胞以及不正常嗜酸性白血球中被表現出來。以流式細胞儀來分析芽細胞的免疫型，發現它們具有朝向顆粒化分化之免疫型。骨髓切片檢查發現過多的細胞，其中有40%的芽細胞，免疫組織化染色顯示他們同時表現CD34，CD117 和 MPO。染色體分析發現16號染色體上有inv(16)(p13q22)之基因轉位。這是一個少見的AMML Eo病例，我們採用多種檢驗方法，包含型態學、流式細胞儀以及染色體分析加上細胞化學、免疫組織化染色來確定診斷。(長庚醫學 2006;29:532-7)

關鍵字：急性骨髓性白血病，急性骨髓性白血球，染色體異常，嗜酸性白血球。