Detection of Acute Lymphoblastic Leukemia Involvement in Pleural Fluid in an Adult Patient with Ataxia Telangiectasia by Flow Cytometry Method

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Abstract Ataxia-telangiectasia (AT) is a rare multisystem, neurodegenerative genetic disorder. Patients should be closely monitored due to risk of malignancy development. Due to its wide clinical heterogeneity, it often leads physicians to an inaccurate or missed diagnosis, and insight into this rare disease is important. Pediatric patients may develop lymphomas and acute lymphoblastic leukemia (ALL). However, in adults, there are limited numbers of reports regarding association of AT and ALL. Rarely, ALL cases may present with pleural fluid involvement. In our study, we presented an adult case with AT, in which ALL involvement was detected in pleural fluid by flow cytometry (FC). A 20-years old male presented to emergency department with fever, shortness of breath and cough, as he had been followed with a diagnosis of AT. The following findings were detected in laboratory tests: Hb, 11.5 g/L; WBC, $36 \times 10^9/L$; Plt: $140 \times 10^9/L$. Blastic cells were observed in peripheral blood smear. On chest radiography, pleural fluid appearance was observed. On thorax CT, pleural fluid was detected in both hemithorax. Cytoplasmic CD3(+) and superficial CD3 (+), CD45 (+), CD5 (+), CD7 (+) and CD38 (+) was found in the flow cytometric

evaluation of peripheral blood. Superficial CD3 (+), CD2 (+), CD5 (+) and CD7 (+) were found in flow cytometric evaluation of pleural fluid. These findings were considered as consistent with pleural involvement of T-ALL. FC is a potentially useful diagnostic tool for clinical practice and it is a convenience method which has an important role in detection of ALL in patients with pleural fluid.

Keywords Ataxia-telangiectasia · Acute lymphoblastic leukemia · Flow cytometry · Pleural effusion

Introduction

Ataxia-telangiectasia (AT) is a hereditary disorder characterized by progressive neurological dysfunction, oculocutaneous telangiectasia, immunodeficiency, cancer susceptibility, and radiation sensitivity [1]. The prognosis of the malignancies is impaired by the immunodeficiency and the susceptibility to ionizing radiation and chemotherapeutics. The diagnosis of AT is based on the typical clinical picture: ataxia and telangiectasia. However, an increase in alpha-fetoprotein (AFP) level and the identification of the AT mutated gene (ATM) may help to make an early diagnosis [2–5]. Pediatric patients may develop lymphomas and acute lymphoblastic leukemia (ALL), especially of the T-lineage [6]. In our study, we presented an adult AT case, in which ALL involvement was detected in pleural fluid by FC evaluation.

Case

A 20-years old male presented to emergency department with fever, shortness of breath and cough as he had been

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followed with a diagnosis of AT. In the history, it was found out that he had imbalance in walking since 2 years of age; this difficulty in walking exacerbated around 5 years of age and he became unable to walk at 7 years of age. Bilateral conjunctival telangiectasia was identified in the physical examination of the patient who referred to Pediatrics Department of Erciyes, University Medicine School at 12 years of age. In neurologic examination, cerebellar ataxia and horizontal nystagmus were detected. No pathological reflex was detected. Deep tendon reflexes were hypoactive. On the cranial magnetic resonance imaging (MRI), there was cerebellar atrophy. There was an increase in alpha fetoprotein (AFP; 46.50 IU/mL, range 0–3 IU/mL) in laboratory evaluations. Following findings were recorded in blood tests: Immunoglobulin (Ig) G: 671 mg/dL (range 740–1,450 mg/dL), IgA: 126 mg/dL (range 80–190 mg/dL), IgM: 192 mg/dL (range 76–195 mg/dL), IgE: 3 IU/mL (range 0–100 IU/mL). It was failed to perform genetic evaluation, as it was unavailable in our facility at that period. Intravenous Ig supplementation was given, when needed, by measuring clinical Ig values. Patient, who presented to emergency department with fever, cough and shortness of breath lasting for 2–3 days, had a moderate general status with superficial, tachypneic breathing. He was conscious with vital signs as follows: body temperature 38.5°; heart rate 110 beats per minute; blood pressure 110/70 mmHg. Breath sounds were bilaterally faint at basal regions of lung. On chest radiography, pleural fluid appearance was observed. On thorax CT, pleural fluid was detected in both hemithorax. The following findings were detected in peripheral blood analysis: Hb: 11.5 g/L (range 14–18 g/L); white blood cells (WBC): $36 \times 10^9/L$, (range $4.8\text{--}10.8 \times 10^9/L$); platelet count: $140 \times 10^9/L$ (range $130\text{--}400 \times 10^9/L$). Blastic cells were observed in peripheral blood smear. In the FC evaluation of peripheral blood, cytoplasmic CD3 (+) and superficial CD3 (+), CD45 (+), CD5 (+), CD7 (+) and CD38 (+) were detected in lymphoid series. CD4: CD8 ratio was 99.7:0.3. These findings were consistent with T-ALL (Fig. 1). A pleural tap was performed, which revealed serofibrinous fluid with a protein level of 6.2 g/dL, Hb: 0 g/L, red blood cell (rbc): 0.02 cells/ μL , and white blood cell count of $6.50 \times 10^9/L$ consisting of lymphocytes (80 %) and neutrophils (20 %). FC evaluation of pleural fluid was performed by using FACS Calibur flow cytometer (Becton–Dickinson, Erembodegem, Belgium). Superficial CD3 (+), CD2 (+), CD5 (+) and CD7 (+) were detected in flow cytometric evaluation of pleural fluid (Fig. 2). These findings were considered as consistent with pleural involvement of T-ALL. Galactomannan and tuberculosis were found to be negative in viral evaluations of pleural fluid. Shortness of breath was relieved in patient after removing 500 cc fluids by pleural tap. Bacterial and

mycobacterial culture tests of pleural fluid were reported as negative. In this patient a parenteral chemotherapy protocol consisting of steroids, vincristine, doxorubicin, methotrexate, folinic acid and L-asparaginase in addition to intrathecal methotrexate, cytosine arabinoside and dexamethasone was scheduled.

Discussion

Ataxia-telangiectasia is a rare autosomal recessive disease notable for neurodegeneration, chromosomal instability, and a predisposition to cancer [7, 8]. Because of increased chemosensitivity, the treatment of AT patients with malignancies requires extremely careful planning and caution with respect to the use of chemotherapy [9–11]. ALL, cancer of the white blood cells, is a heterogeneous disease that mainly occurs due to the malignant cloning of lymphocytes. T-ALL constitutes approximately 25 % of all adult cases of ALL. Our case is also in high risk group due to AT and was diagnosed as T-ALL at 20 years of age. Flow cytometry (FC) has been widely used in the diagnosis in addition to other diagnostic modalities. Advances in cellular immunology have made immunophenotype analysis by FC an essential tool in the diagnosis and classification of ALL, in that it is now far easier to distinguish the source and differentiation stages of ALL accurately, providing a reference point for clinical treatment [12–14]. The immunophenotyping of ALL by FC is based on the detection of surface and internal differentiation. The immunologic phenotype of T cells gradually changes during differentiation, finally resulting in mature T cells that express the CD3 antigen on their cell surface [15]. T cell markers are CD1a, CD2, CD3 (surface and cytoplasm), CD4, CD5, CD7 and CD8. Recent studies showed that the diagnosis of T-ALL rests on the demonstration of cytoplasmic and superficial CD3 [16, 17]. In our study, in addition the CD4 (+), CD5 (+), CD7 (+), cytoplasmic and surface CD3 (+) were detected. Studies evaluating the usefulness of FC in serous cavity effusions are few [18]. However, FC performed with serous fluids has advantages such as convenience in use, rapid results and low costs. FC is an effective method for the characterization of cancer cells in clinical effusion specimens in both the diagnostic and research settings, and that this method is comparable to immunohistochemistry in terms of sensitivity and specificity, with the additional advantage of providing quantitative data [19, 20]. In our case, ALL diagnosis and pleural involvement were supported by FC analysis in both peripheral blood and pleural fluid. Nearly all hematologic malignancies can occasionally present with or develop pleural effusions during the clinical course of disease. But acute leukemias are rarely accompanied by pleural

Fig. 1 Findings of flow cytometry in peripheral blood **Fig. 2** Findings of flow cytometry in pleural fluid

involvement [21]. In cases of hematologic pleural effusions, drug toxicity, underlying infectious, secondary malignant or rarely autoimmune causes should be carefully sought. In our case, ALL involvement was identified as the cause of pleural fluid collection by FC. In most cases, the pleural fluid responds to treatment of the primary disease, whereas resistant or relapsing cases may necessitate pleurodesis. In our case, pleural tap was performed in order to remove the pleural fluid which was followed by relief in

shortness of breath. No pleurodesis was required with the scheduled chemotherapy protocol in the follow-up.

Flow cytometry is frequently used as an ancillary technique for the diagnosis of hematological malignancies and it has become the most important method for diagnosis and typing of ALL. Furthermore, it is a method that can contribute in management of malignancies as it is widely available, which can be used in analysis of all other body fluids as well as peripheral blood and bone marrow.

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