증례보고
Lab Med Online
Vol. 8, No. 1: 24-28, January 2018
https://doi.org/10.3343/lmo.2018.8.1.24

진단혈액학 LMO

급성전골수구백혈병 환자의 가슴막액에서 *PML-RARA* 양성 전골수구가 발견된 예

Leukemic Pleural Effusion in Acute Promyelocytic Leukemia: A Case Report

황나래 · 노숭기 · 함지연 · 서장수
Narae Hwang, M.D., Soongki Roh, M.D., Ji Yeon Ham, M.D., Jang Soo Suh, M.D.

경북대학교 의과대학 임상병리학교실
Department of Clinical Pathology, Kyungpook National University School of Medicine, Daegu, Korea

In patients with acute myeloid leukemia (AML), pleural effusion may be attributed to various factors, including infection, hypoalbuminemia, and renal failure. However, leukemic infiltration of the pleural fluid is rarely reported and poorly understood. Extramedullary diseases have been reported with increasing frequency as the survival rates of patients with AML have increased. However, the reported prognostic effects of leukemic pleural effusion in patients with AML range from none to a worse prognosis. Here, we report a case of acute promyelocytic leukemia (APL) in a patient exhibiting leukemic pleural effusion with fluorescence *in situ* hybridization (FISH) results indicating the presence of the *PML-RARA* fusion gene. A 52-year-old man presented with pancytopenia, dyspnea, and fever. He had a medical history of hypertension, end-stage renal disease, and hepatitis B virus-related liver cirrhosis. A peripheral blood smear revealed the presence of multiple abnormally hypergranular promyelocytes. White blood cell differential counts were not performed due to severe pancytopenia. A bone marrow examination, immunophenotyping analysis, and cytogenetic and molecular studies revealed APL. The patient was treated with all-trans retinoic acid immediately after abnormal promyelocytes were observed in the peripheral blood smear, but induction chemotherapy was delayed because of his poor condition. His persistent dyspnea and abdominal discomfort led to a thoracentesis and the observation of abnormal promyelocytes that were positive for *PML-RARA* fusion gene by FISH. To our knowledge, this is the first report of leukemic pleural infiltration with *PML-RARA* fusion gene-positivity via FISH.

Key Words: Acute promyelocytic leukemia, Leukemic pleural effusion, *PML-RARA*

INTRODUCTION

The recent increases in survival rates among patients with acute myeloid leukemia (AML) have been accompanied by increasingly frequent reports of extramedullary disease (EMD). EMD, with an overall incidence of 2.5–30%, has mainly been reported in AML cases involving myelomonocytic or monoblastic disease and those with recurrent genetic abnormalities such as t(8;21) and inv(16) [1-4]. The prognosis for EMD varies among reports from no effect to an unfavorable clinical outcome [2, 5, 6].

In AML, pleural effusion may be attributed to various factors, including infections, hypoalbuminemia, and renal failure. By contrast, leukemic infiltration of the pleura is rarely reported and poorly understood. Only a few reports have described leukemic pulmonary infiltration in the extramedullary relapse of M2, M3, and M5 French-American-British subtypes of AML (Table 1) [4, 7-10]. A few reports of extramedullary relapse in acute promyelocytic leukemia (APL) have revealed that the central nervous system (CNS) was the most common extramedullary site, followed by the skin [11, 12]. However, reports of leukemic pleural infiltration in the M3 subtype at initial presentation are scarce [4, 7]. Herein, we report a rare case of APL, with leukemic pleural effusion that was identified as positive for the *PML-RARA* fusion gene via *in situ* hybridization (FISH).

Corresponding author: Jang Soo Suh
Department of Clinical Pathology, Kyungpook National University School of Medicine, 680 Gukchebosang-ro, Chung-gu, Daegu 41944, Korea
Tel: +82-53-200-5293, Fax: +82-53-200-3367, E-mail: suhjs@knu.ac.kr

Received: June 15, 2017
Revision received: September 26, 2017
Accepted: October 26, 2017

This article is available from http://www.labmedonline.org

Table 1. Reported cases of pleural infiltration in AML

Case No.	Age/Sex	WBC ($\times 10^9$/L)	Hb (g/dL)	Platelet ($\times 10^9$/L)	FAB	BM*	FCM	FISH or RT-PCR	EMD	P	R
1	34/M	NA	NA	NA	M3	N	ND	RT-PCR *PML-RARA* (+)†	Pleura, heart, pericar-dium	D	[7]
2	39/M	4.52	14	212	M3	N	PF	ND	Pleura	CR	[4]
3	53/M	11.5	8	103	M2	Y	ND	FISH *RUNX1-RUNX1T1* (+)	Pleura	CR	[8]
4	19/M	6.9	9.5	94	M5	Y	BAL	ND	BAL, CSF	CR	[9]
5	4/M	NA	NA	NA	M2	Y	ND	FISH Lung Bx: *RUNX1-RUNX1T1*(+)	Lung	CR	[10]

*BM involvement; †*PML-RARA* (+) in peripheral blood
Abbreviations: N, No; Y, Yes; ND, not done; NA, not available; CR, complete remission; FCM, flow cytometry; P, prognosis; R, reference; D, dead; PF, pleural fluid; BAL, bronchoalveolar lavage; EMD, extramedullary disease; FAB, French-American-British classification; Bx, biopsy.

CASE

A 52-year-old man visited the emergency room of our institution presenting with dyspnea and fever. He had a medical history of hypertension, end-stage renal disease, and hepatitis B virus-related liver cirrhosis. He was on continuous ambulatory peritoneal dialysis. Laboratory examinations indicated pancytopenia with the following measurements: Hb, 6.4 g/dL; leukocyte count, 0.9×10^9/L; and platelet count, 24×10^9/L. A peripheral blood smear showed multiple abnormally hypergranular promyelocytes. White blood cell differential counts were not performed due to severe pancytopenia. A bone marrow aspirate smear revealed increased cellularity, comprised of 56.3% abnormal promyelocytes with Auer rod bundles (Fig. 1A). The cells were strongly positive for peroxidase and negative for periodic acid–Schiff staining. Flow cytometric analysis revealed a typical APL immunophenotype, with strong surface expression of CD13, CD33, and CD117 and cytoplasmic myeloperoxidase expression, but no CD34 or HLA-DR expression. The 47,XY,+add(5)(q11.2)x2,der(5;8)(q10;p10),del(7)(q32), t(15;17)(q22;q21) chromosome complement was observed in all 21 metaphase cells (Fig. 1B). Real-time quantitative reverse transcription PCR for *PML-RARA* indicated a *PML-RARA/ABL* ratio of 0.553. *KIT* and *FLT3* mutation analyses were negative. Laboratory examinations revealed azotemia with the following measurements: blood urea nitrogen, 69.5 mg/dL (reference ranges 6.0–20.0); creatinine, 14.09 mg/dL (0.7–1.3); erythrocyte sedimentation rate, 112 mm/h (0–10); C-reactive protein (CRP), 2.88 mg/dL (<0.5); procalcitonin, 0.804 ng/mL (0–0.1); prothrombin time, 13.5 seconds (10–14); and activated partial thromboplastin time, 31.6 seconds (20–40). The patient was treated with all-trans retinoic acid (ATRA) immediately after detection of abnormal promyelocytes in the peripheral blood smear, but induction chemotherapy was delayed because

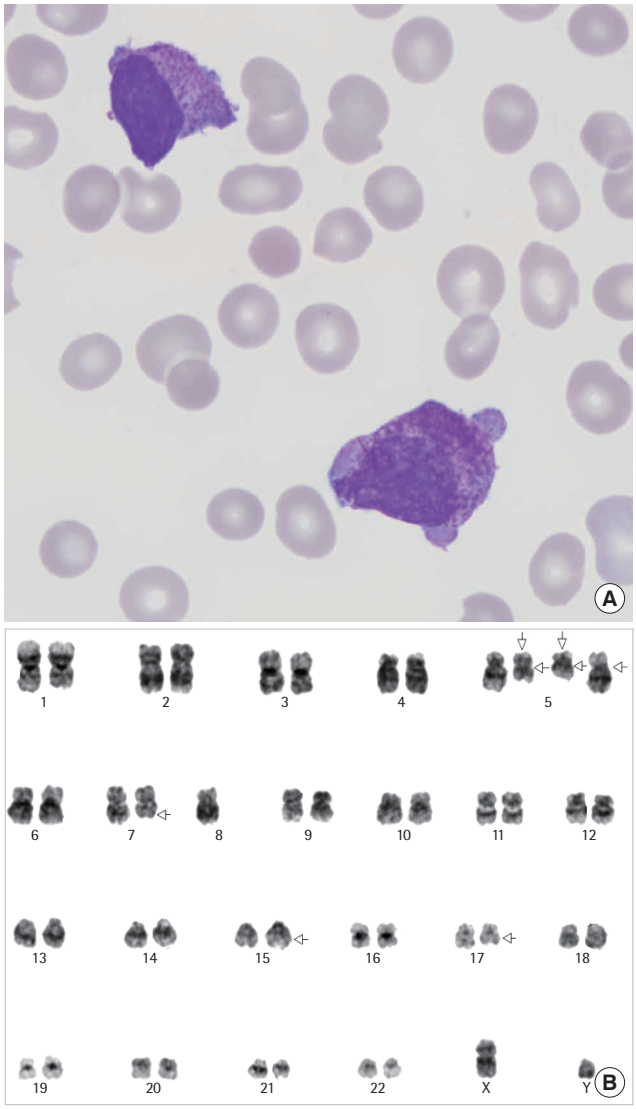

Fig. 1. Abnormal promyelocytes with multiple Auer rods in the bone marrow (Wright's stain ×1,000) (A) and conventional bone marrow chromosome analysis result showing a 47,XY,+add(5)(q11.2)x2,der(5;8)(q10;p10),del(7)(q32), t(15;17)(q22;q21) [21] karyotype (B).

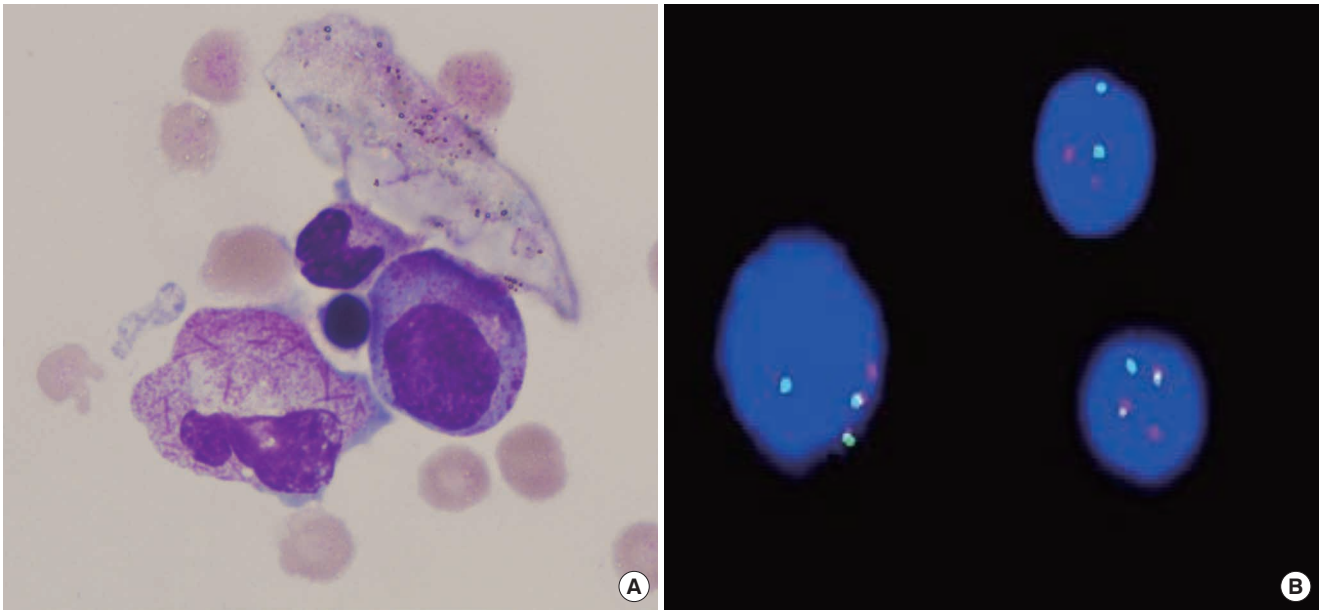

Fig. 2. Abnormal promyelocytes with multiple Auer rods in pleural fluid (Wright's stain ×1,000) (A) and dual-color, dual-fusion fluorescence *in situ* hybridization analysis on interphase cells showing two green signals, indicating the t(15;17) translocation (B).

of his poor condition. On the ninth day after admission, the patient complained of severe dyspnea and arthralgia and chest x-ray results suggested pleural effusion. Accordingly, an immediate thoracentesis was performed. The pleural fluid was serous and exudative, with the following parameters: protein, 4.0 g/dL; glucose, 216 mg/dL; and lactate dehydrogenase, 360 IU/L. A cytospin analysis revealed abnormal promyelocytes containing multiple Auer rods (Fig. 2A) and interphase FISH of the pleural fluid indicated the presence of the *PML-RARA* fusion gene (Fig. 2B). The patient began AIDA (ATRA+idarubicin) induction chemotherapy and the pleural effusion resolved rapidly, according to a chest x-ray. Further complaints of dyspnea and arthralgia suggested retinoic acid syndrome and the patient was switched from ATRA to dexamethasone (10 mg, three times a day for 10 days). ATRA was restarted when the patient's condition improved after dexamethasone therapy. On the eighth day in the hospital, *Aspergillus* antigen was detected in his serum and amphotericin B was administered intravenously. Repeated tests for *Aspergillus* antigen were negative after 20 days of amphotericin B therapy. Pleural fluid and blood culture findings remained negative for other fungi, bacteria, and viruses. However, on the twenty-third day in the hospital, he developed a fever, with an increased CRP level (20.95 mg/dL; normal is <0.5) and was intravenously administered empirical antibiotics, including meropenem, vancomycin, and fluconazole. Despite the combined administration of ATRA, chemotherapy, and supportive care, his uremia and general condition became worse and he refused aggressive therapies such as renal replacement therapy, mechanical ventilation, and intensive care unit admission. He died in the hospital on day 37 of shock and multi-organ failure.

DISCUSSION

PML-RARA-positive APL has the most favorable prognosis among all AML subtypes because the differentiating agent, ATRA, when used in combination with anthracycline, can induce complete remission in most patients [13]. Pulmonary involvement is a rare complication of AML, and only a few reports have described this phenomenon [4, 7-10]. Azoulay and colleagues reported 20 cases of acute monocytic leukemia involving acute respiratory failure related to leukemic pulmonary involvement from leukostasis or leukemic infiltration [14]. In their case series, all patients developed respiratory problems after initiation of chemotherapy, with 50% of the patients dying during chemotherapy. Therefore, the authors suggested that early invasive diagnostic and therapeutic management along with intensive care unit admission before chemotherapy initiation is recommended for all patients with any degree of respiratory impairment [14]. This strategy will be helpful for patients

https://doi.org/10.3343/lmo.2018.8.1.24

because radiographic features of leukemic pulmonary infiltration are known to be heterogeneous and can be difficult to differentiate from other non-infectious causes such as hemorrhage and acute respiratory distress syndrome [15]. Others reported a case of granulocytic sarcoma that was initially mistaken for pneumonia in a patient with t(8;21)(q22;q22). This chromosomal translocation formed the *RUNX1-RUNX1T1* fusion gene, causing AML. In this patient, the bronchoalveolar lavage (BAL) fluid contained 71% myeloblasts, and the abnormal chest radiograph findings did not change despite the administration of empirical broad-spectrum antibiotics. However, the x-ray findings resolved after systemic chemotherapy [10].

Currently, the skin and central nervous system (CNS) are the most common sites of EMD in patients with APL, followed by the mediastinum, gingiva, and auditory canal. By contrast, pulmonary involvement in APL is very rare. Botton and colleagues analyzed 740 APL cases in which only ten (1.35%) patients relapsed with EMD, most with CNS relapses and none with pulmonary EMD [16]. Only two previous reports described APL with EMD in the pleura. In one report, the patient exhibited lung infiltration with pleural effusion after achieving complete remission in the bone marrow. This patient was diagnosed with extramedullary relapse via immunophenotypic analysis of pleural fluid [4]. In another report, the patient relapsed in pleura, heart, and pericardium after allogeneic stem cell transplantation (SCT), with positive *PML-RARA* transcripts detected by RT-PCR [7]. However, karyotype and FISH analyses were not performed to prove clonality in both cases. Our patient exhibited a pleural effusion, which was detected by chest x-ray on his ninth day in the hospital, and this effusion resolved after the initiation of AIDA chemotherapy. Although a lung biopsy or BAL was not performed in the present case, the leukemic pleural infiltration was likely present at an early stage of the disease. Furthermore, FISH analysis has been used to rule out other confusing cases of leukemic pleural effusion or granulocytic sarcoma of the lung and could, therefore, be a useful tool for the diagnosis of leukemic infiltration in other tissues [8, 10].

Although the mechanism underlying the development of pulmonary extramedullary manifestations of leukemia has not been established, interactions involving direct endothelial cell damage by aggregates of circulating leukemic cells have been proposed [17]. Additionally, Ko and colleagues suggested that ATRA could modulate the expression of intercellular adhesion molecule-1 (ICAM-1) and other adhesion molecules in APL cells, exacerbating the ability of leukemic cells to infiltrate into extramedullary tissues and cause EMD [18].

In the present case, *Aspergillus* antigen was detected in the patient's serum the day before the abnormal promyelocytes were observed in his pleural fluid. However, the pleural fluid and blood cultures were repeatedly negative for other fungi, bacteria, and viruses and a repeated *Aspergillus* antigen test was negative after amphotericin B administration. Therefore, the pleural effusion observed in this patient is likely attributable to leukemic pleural infiltration, rather than an infectious agent.

In the present case, multiple comorbidities such as end-stage renal disease, liver cirrhosis, the lack of early disease management, and the refusal of therapy contributed to the rapid demise of the patient. Although there have been a few reports of leukemic pleural infiltration in relapsed APL, this is the first report to document a pleural fluid infiltration of APL at the initial presentation that tested positive for the *PML-RARA* fusion gene by FISH.

요 약

급성골수성백혈병 환자에서 가슴막삼출의 원인은 감염, 저알부민혈증, 신부전 등으로 다양하나 백혈병 세포가 가슴막액에 침범한 사례는 드물게 보고되고 있다. 급성골수성백혈병에서 생존률이 증가하면서 골수외백혈병의 사례가 증가하고 있으나 이의 예후에 대한 영향은 예후와 관계없다는 보고부터 더 좋지 않은 예후까지 다양하게 보고되고 있다. 저자들은 급성전골수구백혈병 환자의 가슴막액에서 전골수구가 관찰되었고 *PML-RARA* 유전자가 형광제자리부합법으로 확인된 케이스를 보고하는 바이다. 52세 남자가 범혈구감소증, 호흡곤란, 발열을 주소로 응급실에 내원하였고 이 환자는 기저질환으로 고혈압, 말기신질환, B형 간염 바이러스로 인한 간경화가 있는 환자였다. 말초혈액도말검사에서 다수의 비정상적인 과과립성의 전골수구가 관찰되었고 백혈구 감별계산은 심한 범혈구감소증 때문에 실시하지 않았다. 골수흡인검사, 면역표현형검사, 세포유전학, 분자유전학검사에서 급성전골수구백혈병이 확인되었다. 환자는 말초혈액도말검사에서 비정상적인 전골수구가 확인된 직후 ATRA 치료를 바로 시작하였으나 유도항암요법은 환자의 전신상태가 취약하여 연기되었다. 환자가 지속적인 호흡곤란과 복부팽만감을 호소하여 가슴천자술이 시행되었고 가슴막액에서 Auer rod를 가지는 전골수구가 관찰되었고 형광제자리부합법으로 *PML-RARA* 유전자가 확인되었다. 본 사례는 급성전골수구백혈병 환자의 가슴막액에서 *PML-RARA* 유전자 양성인

전골수구가 발견된 첫 번째 사례이다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Muss HB and Moloney WC. Chloroma and other myeloblastic tumors. Blood 1973;42:721-8.
2. Chang H, Brandwein J, Yi QL, Chun K, Patterson B, Brien B. Extramedullary infiltrates of AML are associated with CD56 expression, 11q23 abnormalities and inferior clinical outcome. Leuk Res 2004;28:1007-11.
3. Tallman MS, Kim HT, Paietta E, Bennett JM, Dewald G, Cassileth PA, et al. Acute monocytic leukemia (French-American-British classification M5) does not have a worse prognosis than other subtypes of acute myeloid leukemia: a report from the Eastern Cooperative Oncology Group. J Clin Oncol 2004;22:1276-86.
4. Disel U, Yavuz S, Paydas S, Sahin B, Zeren H. Extramedullary relapse in the pleura in acute promyelocytic leukemia. Leuk Lymphoma 2003;44:189-91.
5. Ganzel C, Manola J, Douer D, Rowe JM, Fernandez HF, Paietta EM, et al. Extramedullary disease in adult acute myeloid leukemia is common but lacks independent significance: analysis of patients in ECOG-ACRIN Cancer Research Group trials, 1980-2008. J Clin Oncol 2016;34:3544-53.
6. Kobayashi R, Tawa A, Hanada R, Horibe K, Tsuchida M, Tsukimoto I. Extramedullary infiltration at diagnosis and prognosis in children with acute myelogenous leukemia. Pediatric Blood Cancer 2007;48:393-8.
7. Nasilowska-Adamska B, Majewski M, Seferynska I, Szczepinski A, Tomaszewska A, Prochorec-Sobieszek M, et al. Predictive value of RT-PCR PML-RARA transcript monitoring for extramedullary relapse of acute promyelocytic leukemia in the pleura, heart and pericardium after allogeneic SCT. Ann Transplant 2007;12:33-8.
8. Ou MC, Hwang WL, Teng CL. Leukaemic pleural effusion in acute myeloid leukaemia. Br J Haematol 2011;154:669.
9. Hoffman LM, Gore L, Maloney KW. Pulmonary presentation of relapsed acute myeloid leukemia. J Pediatr Hematol Oncol 2014;36:228-30.
10. Lee DA, Harris CP, Gresik VM, Rao P, Lau CC. Granulocytic sarcoma presenting as pneumonia in a child with t(8;21) acute myelogenous leukemia: diagnosis by fluorescent in situ hybridization. J Pediatr Hematol Oncol 2004;26:431-4.
11. Ganzel C and Douer D. Extramedullary disease in APL: a real phenomenon to contend with or not? Best Pract Res Clin Haematol 2014;27:63-8.
12. Vega-Ruiz A, Faderl S, Estrov Z, Pierce S, Cortes J, Kantarjian H, et al. Incidence of extramedullary disease in patients with acute promyelocytic leukemia: a single-institution experience. Int J Hematol 2009;89:489-96.
13. Swerdlow SH, Campo E, et al. eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC, 2008:113-4.
14. Azoulay É, Fieux F, Moreau D, Thiery G, Rousselot P, Parrot A, et al. Acute monocytic leukemia presenting as acute respiratory failure. Am J Respir Crit Care Med 2003;167:1329-33.
15. Potenza L, Luppi M, Morselli M, Tonelli S, D'apollo N, Facchini L, et al. Leukaemic pulmonary infiltrates in adult acute myeloid leukaemia: a high-resolution computerized tomography study. Br J Haematol 2003;120:1058-61.
16. De Botton S, Sanz MA, Chevret S, Dombret H, Martin G, Thomas X, et al. Extramedullary relapse in acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Leukemia 2006;20:35-41.
17. Porcu P, Cripe LD, Ng EW, Bhatia S, Danielson CM, Orazi A, et al. Hyperleukocytic leukemias and leukostasis: a review of pathophysiology, clinical presentation and management. Leuk Lymphoma 2000;39:1-18.
18. Ko BS, Tang JL, Chen YC, Yao M, Wang CH, Shen MC, et al. Extramedullary relapse after all-trans retinoic acid treatment in acute promyelocytic leukemia-the occurrence of retinoic acid syndrome is a risk factor. Leukemia 1999;13:1406-8.

https://doi.org/10.3343/lmo.2018.8.1.24