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Case Study

Primary Pulmonary Classical Hodgkin Lymphoma with Two Recurrences in the Mediastinum : A Case Report

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We report a case of primary pulmonary classical Hodgkin lymphoma (CHL) in a 58-year-old woman. Twelve years ago, the patient complained of slight fever and weight loss. A mass of about 5 cm in diameter was seen in the right lung on radiography and computed tomography (CT). Right total pneumonectomy and resection of mediastinal lymph nodes were performed. A pathological examination led to a strong suspicion of Hodgkin disease (HD) (now referred to as CHL), but a definite diagnosis could not be made at the time. Six years later, a chest CT showed a tumor around the ascending aorta, which was treated successfully by radiation therapy. Six years later, the chest CT revealed a tumor in the anterior mediastinum. CHL was diagnosed based on an immunohistochemical re-examination of lung specimens resected 12 years earlier and CT-guided fine needle tumor biopsy specimens of the second recurrent tumor in the anterior mediastinum were compatible with the recurrence of CHL. Therefore, we diagnosed this case as primary pulmonary CHL that later relapsed in the mediastinum. The tumor size was reduced by radiation therapy and the patient is currently under observation as an outpatient. 〔*J Clin Exp Hematopathol 50(2) : 151-157, 2010*〕

Keywords: classical Hodgkin lymphoma, immunohistochemistry, primary pulmonary lymphoma, recurrence

INTRODUCTION

Primary pulmonary classical Hodgkin lymphoma (CHL) is a rare disease with fewer than 100 reported cases. Here, we describe a case of CHL that primarily involved the lung and then recurred twice in the mediastinum.

CASE REPORT

Clinical summary

The patient was a 58-year-old woman who first presented twelve years ago with slight fever and weight loss. Her past history included acute hepatitis at 19 years old and appendicitis at 30 years old. Superficial lymphadenopathy was not detected upon physical examination. Her peripheral blood cell count was normal. Other laboratory data indicated slight elevation of C-reactive protein (3.9 mg/dL), but were otherwise unremarkable. Tumor markers including carcinoembryonic antigen, squamous cell carcinoma antigen (SCC-Ag), cytokeratin 19 fragment antigen (CYFRA), and neuron-specific enolase were negative. However, a chest radiograph revealed a mass in the middle of the right lung (Fig. 1). Computed tomography (CT) showed an irregular mass of about 5 cm in diameter with a cavity in the right lung S3, but no hilar or mediastinal lymphadenopathy, and mediastinal infiltration was not detected (Fig. 2).

Bronchoscopy was performed and a histological examination revealed granulation tissue only. Cytological specimens obtained by transbronchial brushing revealed scattered large tumor cells with prominent nucleoli (Fig. 3). The diagnosis was positive for malignant cells and adenocarcinoma was suspected. Right total pneumonectomy and resection of mediastinal lymph nodes were performed three months after the first presentation. Upon pathological examination, Hodgkin disease (HD) (now referred to as CHL) was strongly suspected, but a definite diagnosis beyond malignant lymphoma could not be made at that time. After the operation, the patient was placed under observation without chemotherapy

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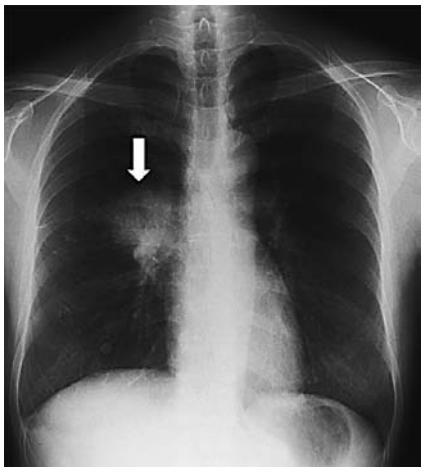

Fig. 1. Chest radiography showing a mass (*arrow*) in the middle of the right lung.

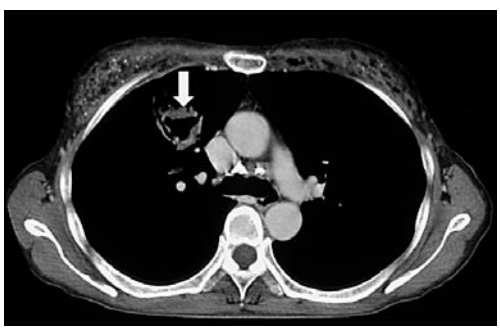

Fig. 2. Chest computed tomography showing a mass of about 5 cm in diameter with a cavity (*arrow*) in the right lung S3.

at her request. Six years later, she suffered from hoarseness and chest CT showed a tumor around the ascending aorta. The tumor was suspected to be a recurrence of malignant lymphoma and chemotherapy was under consideration. However, she rejected chemotherapy and radiation therapy was selected. The tumor disappeared after radiation therapy, but after another six years chest CT showed a tumor in the anterior mediastinum. CT-guided fine needle tumor biopsy and re-examination of lung specimens resected 12 years earlier were performed. CHL was diagnosed based on an immunohistochemical re-examination of lung specimens. Further, CT-guided fine needle tumor biopsy specimens of the second recurrent tumor in the anterior mediastinum were compatible with the recurrence of CHL. Radiation therapy was used again because she rejected chemotherapy again, and the tumor was reduced in size. The patient is currently under observation as an outpatient.

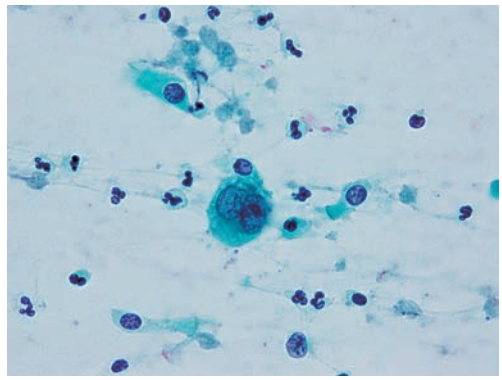

Fig. 3. A bronchial abrasive cytology specimen showing scattered large cells with prominent nucleoli and background infiltration of lymphocytes and neutrophils. Papanicolaou's stain, ×1,000.

Pathological findings

1) Cytology

A bronchial abrasive cytology specimen showed scattered large cells with prominent nucleoli and background infiltration of lymphocytes and neutrophils. The diagnosis was positive for malignant cells and adenocarcinoma was suspected (Fig. 3).

2) Histology

The resected right lung included a necrotic tumor mass of about 5 cm in diameter in the upper lobe (Fig. 4). Morphological findings were obtained using H&E staining of formalin-fixed, paraffin-embedded specimens of the lung from right pneumonectomy samples (Fig. 5) and from CT-guided fine needle biopsy samples from the anterior mediastinal tumor that were collected 12 years later. The lung specimens showed a necrotic area with both mononucleated and multinucleated giant cells similar to Hodgkin and Reed-Sternberg (HRS) cells in the tumorous lesion (Fig. 6a). Although there were only a few typical lacunar HRS cells, a background infiltrate of eosinophils, small lymphocytes and bands of fibrosis encircling were present. These histological findings suggested HD, which is now referred to as nodular sclerosis classical Hodgkin lymphoma (NSCHL). However, 12 years ago, the tumor cells were not clearly positive for CD30 or CD15 immunohistologically, and so a definite diagnosis could not be made. There was no evidence of involvement of the regional and mediastinal lymph nodes. Twelve years later, specimens collected by CT-guided fine needle biopsy of the tumor in the anterior mediastinum showed some large cells with infiltration of a few small lymphocytes and eosinophils, and fibrous proliferation (Fig. 6b).

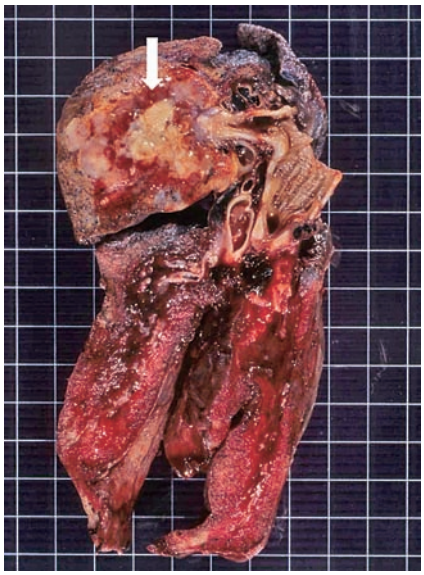

Fig. 4. A necrotic tumor mass of about 5 cm in diameter (*arrow*) in the upper lobe of the resected right lung.

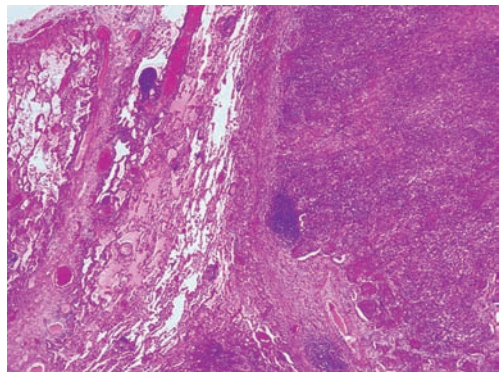

Fig. 5. H&E-stained specimens of the lung from right pneumonectomy at low-power magnification (×100), showing a lesion with infiltration of lymphocytes.

Immunohistochemistry and Epstein-Barr virus (EBV)-encoded small RNA in situ *hybridization*

Immunohistochemical analysis and detection of Epstein-Barr virus (EBV)-encoded small RNA by *in situ* hybridization were performed using lung specimens from right total pneumonectomy and samples from CT-guided fine needle biopsy of the recurrent tumor in the anterior mediastinum. The results for these two types of specimens were compared. Formalin-fixed, paraffin-embedded specimens were used for immunohistochemical analysis with a panel of monoclonal antibodies (Table 1). Sections were subjected to either autoclave antigen retrieval in citric acid buffer at pH7.0 for 15 min at 121°C or to microwave antigen retrieval in citric acid buffer at pH7.0 for 30 min at 98°C ; or were incubated with 0.04% proteinase K (Dako Cytomation, Glostrup, Denmark) for 5 min. Endogenous peroxidase was blocked with 3% H_2O_2 for 5 min. After washing with phosphate-buffered saline (PBS), sections were incubated with a primary monoclonal antibody for 60 min at room temperature. After another washing with PBS, sections were incubated using an Envision Kit/HRP (Dako Cytomation). Detection of EBV-encoded small RNA (EBER) by *in situ* hybridization was performed on formalin-fixed, paraffin-embedded specimens of both the lung and the tumor in the anterior mediastinum, using a commercial fluorescein-conjugated peptide nucleic acid (PNA) probe for EBV (EBER PNA probe) and a PNA ISH Detection Kit (Dako Cytomation).

In immunohistochemical analysis, the specimens of the initial lung tumor included tumor cells that were positive for CD30 (Fig. 6c) and CD15, but negative for CD20 and CD3. Immunostaining of PAX5 was weaker for the tumor cells than for reactive B cells (Fig. 6e). Only a few tumor cells were positive for Bob1, but they were negative for Oct-2. The bands of sclerotic collagen divided the cellular infiltration into multiple nodules, but immunostaining for CD21, a follicular dendritic cell marker, did not make the meshwork of the follicular dendritic cells in the nodules. These findings are compatible with CHL and this case was diagnosed as NSCHL. Immunostaining of the large cells in the specimens from the second recurrent mediastinal tumor gave similar results to those for the initial lung tumor (Fig. 6d, 6f). The *in situ* hybridization assay showed that the nuclei of the tumor cells were positive for EBER in specimens from the lung tumor (Fig. 6g) and the mediastinal tumor (Fig. 6h). The specimens of the tumor in the anterior mediastinum were too small and degenerated to determine the subtype of CHL. However, given the results of other immunohistological stainings, we excluded other tumor such as carcinoma and the tumor was considered the recurrence of CHL.

Differential diagnosis

CHL cases rich in neoplastic cells may resemble anaplastic large cell lymphoma (ALCL). In this case, the tumor cells were positive for CD15 and weakly positive for PAX5, and negative for EMA, ALK and T-cell markers such as CD3. Moreover, the detection of EBV-encoded RNA (EBER) was indicative of CHL. Therefore, we were able to distinguish this case from ALCL. We also excluded B-cell lymphoma, including T cell/histiocyte-rich large B-cell lymphoma (THRLBCL) and primary mediastinal (thymic) large B-cell lymphoma (PMBL). In THRLBCL, the large atypical cells express pan B-cell markers and no expression of CD15 and CD30 is found. PMBL expresses B-cell antigens such as

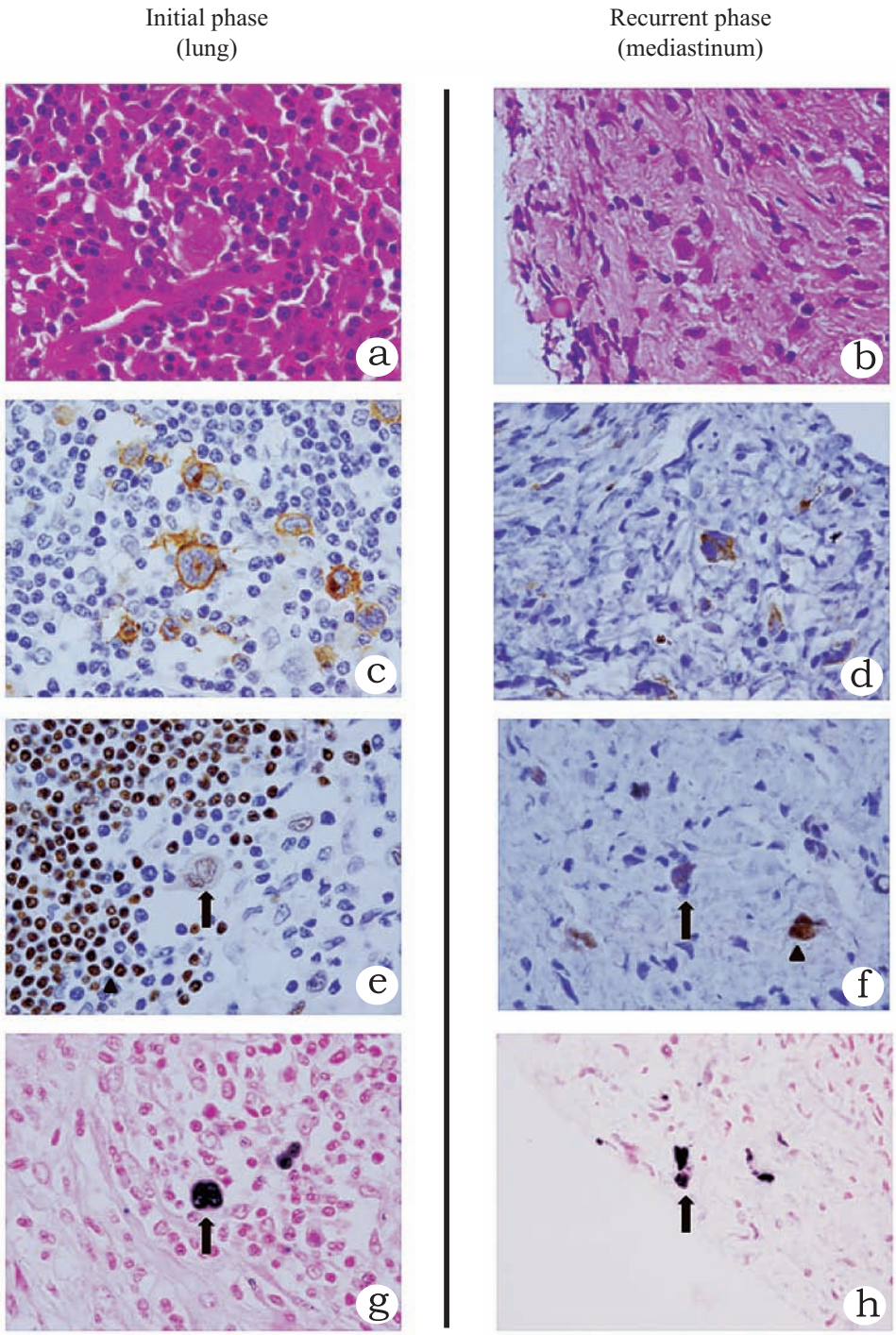

Fig. 6. Re-examination of the initial tumor in the lung (6a, 6c, 6e and 6g) and examination of the recurrent tumor in the anterior mediastinum (6b, 6d, 6f and 6h) by light microscopy at high magnification. (***6a***) H&E-stained lung specimens showed the presence of mononucleated and multinucleated giant cells similar to Hodgkin and Reed-Sternberg (HRS) cells in the tumorous lesion, with background infiltration of eosinophils, small lymphocytes, and sclerosis. ×1,000. (***6b***) H&E-stained specimens of the recurrent tumor showed some large cells, infiltration of small lymphocytes and eosinophils, and fibrous proliferation. ×1,000. (***6c, 6d***) Immunohistologically, the tumor cells were positive for CD30. Counterstained with hematoxylin, ×1,000. (***6e, 6f***) Immunostaining of tumor cells for PAX5 (*arrows*) was weaker than that of reactive B cells (*arrowheads*). Counterstained with hematoxylin, ×1,000. (***6g, 6h***) *In situ* hybridization showed that the tumor cells were positive for Epstein-Barr virus (EBV)-encoded small RNA (EBER) (*arrows*). Counterstained with Kernechtrot, × 1,000.

Table 1. Immunohistochemistry and *in situ* hybridization results for the initial (lung) and recurrent (mediastinum) tumors

Antigen	Clone	Source	Dilution	Retrieval	Immunopositivity	
					Initial phase* (lung)	Recurrent phase (mediastinum)
LCA	2B11+PD7/26	Dako Cytomation, Glostrup, Denmark	1:100	(−)	−	−
CD20	L26	Dako Cytomation, Glostrup, Denmark	1:100	(−)	−	−
CD79a	JCB117	Dako Cytomation, Glostrup, Denmark	1:50	Microwave	−	−
CD45R0	UCHL-1	Dako Cytomation, Glostrup, Denmark	1:100	(−)	−	−
CD3	PS1	Nichirei, Tokyo, Japan	Prediluted	Microwave	−	−
CD5	4C7	Novocastra, Newcastle upon Tyne, UK	1:50	Microwave	−	−
CD15	MCS-1	Nichirei, Tokyo, Japan	Prediluted	Autoclave	+	+
CD30	1G12	Novocastra, Newcastle upon Tyne, UK	Prediluted	Microwave	+	+
PAX5	24	BD Transduction Laboratories, San Jose, CA, USA	1:40	Autoclave	+ (weak)	+ (weak)
Bob1	Polyclonal	Santa Cruz Biotechnology, INC Santa Cruz, CA, USA	1:2000	Autoclave	+ (focal)	−
Oct-2	Polyclonal	Santa Cruz Biotechnology, INC Santa Cruz, CA, USA	1:500	Autoclave	−	−
AE1/AE3	AE1 and AE3	Dako Cytomation, Glostrup, Denmark	1:50	Proteinase K	−	−
EBER	(PNA probe)	Dako Cytomation, Glostrup,			*In situ* hybridization positivity	
		Denmark			+	+

+, positive ; − , negative ; EBER, Epstein-Barr virus (EBV) encoded small RNA
*, the results of re-examination 12 years after the initial presentation

CD20 and CD79a. CD30 is present in more than 80% of cases, but is usually weak and heterogeneous compared to HL. Therefore, the absence of CD20 expression and the presence of CD15 expression and EBV favored diagnosis of CHL in our case.

DISCUSSION

Primary pulmonary lymphomas are uncommon, comprising less than 1% of lung cancers and fewer than 1% of malignant lymphomas, and accounting for only 3.6% of extranodal lymphomas.[1] Primary pulmonary CHL is extremely rare, with Nakachi *et al*.[2] finding only 84 reported cases, including 3 in Japan.[3-5] Kern *et al*.[6] suggested the following criteria for diagnosis of primary pulmonary HD (CHL) : (1) diagnosis by pathological examination documented by photomicrographs or microscopy, (2) disease predominantly in the lung at the time of the original diagnosis, with or without simultaneous involvement of hilar or mediastinal lymph nodes, and (3) exclusion of cases with evidence of peripheral or other distant lymph node or organ involvement and cases in which the pulmonary tumor appears to be an extension of a mediastinal lesion. In our case, the tumor was predominantly in the lung without involvement of hilar or mediastinal lymph nodes, and there was no evidence of disease at other sites at the time of initial presentation. The pathological findings were compatible with NSCHL, including immunohistological findings, so this case clearly meets the criteria for primary pulmonary CHL.

Radin[7] described 61 worldwide cases of primary pulmonary HD (CHL) reported before 1990. Among these patients, 25 were men and 36 were women (a ratio of 1:1.4), and age ranged from 12 to 82 years old (mean 42.5 years old). Of 23 cases after 1990 reviewed by Nakachi *et al*.,[2] the patients ranged in age from 17 to 60 years old and there was no difference in sex. In both Radin and Nakachi *et al*., the most common clinical complaint was a cough with dyspnea, and B symptoms (i.e. weight loss, fever, and night sweats) were less commonly reported. Some of the patients were asymptomatic. Neoplastic lesions were most commonly found in the upper lobes of the lung unilaterally, and tended to present as a solitary mass or multinodular lesion on radiography, sometimes showing cavitation.[8] Histologically, nodular sclerosis and mixed cellularity subtypes were common.

Histopathological findings are necessary to achieve a definite diagnosis of pulmonary HD (CHL), but biopsies taken during bronchoscopy are rarely conclusive.[9] In Radin,[7] bronchoscopic abnormalities were described in about 50% of the patients, but the procedure was diagnostic in only 1 of 35 cases. Similarly, sputum cytology suggested a malignancy in only 1 of 9 patients. In most cases, diagnosis of HD was made by open lung biopsy or lobectomy. Therefore, pulmonary CHL should be included in differential diagnosis, even when pathological findings from biopsy specimens reveal non-specific inflammation. CHL also must be separated from undifferentiated carcinoma and the use of immunohistochemistry can resolve this problem.[10]

In this case, tumor cells were positive for EBER. Serum anti-EBV antibodies have not been examined. The prevalence of EBV in HRS cells varies according to the histological subtype and epidemiologic factors. The association with EBV is less frequent in NSCHL (10-40%) than other types of CHL.[11] There are only a few reports of primary pulmonary Hodgkin lymphoma[12] and primary pulmonary diffuse large B-cell lymphoma associated with EBV infection.[13] The significance of the EBV positivity in primary pulmonary CHL is still unclear.

Radin[7] also found that the prognosis for pulmonary HD was less favorable than that for nodal HD. Among 38 cases, 5 progressed during therapy and 18 relapsed after a complete response. In contrast, Nakachi *et al*.[2] found that 14 of 23 patients survived and 4 relapsed or died, which suggests that the prognosis of pulmonary CHL is not as poor as that reported by Radin. Improved treatments for CHL may have contributed to the improved prognosis. The treatment of primary pulmonary HD before 1960, in the majority of cases, consisted of complete excision of the tumor with or without postoperative irradiation.[6] After 1960, combination chemotherapy predominated. This change must be attributed to a growing awareness of the systemic nature of the lymphomas and to the development of more effective chemotherapeutic agents.[7] Some reports suggested that HD involving the lung could be treated effectively with radiation alone when the lymphoma was at a limited stage and, in contrast, lesions more widely disseminated throughout the lung may better be treated with combination chemotherapy or combined modality therapy. Yousem *et al*.[14] found beneficial results of systemic chemotherapy and Nakachi *et al*. have suggested that chemotherapy is recommended rather than radiotherapy because of the risk of radiation pneumonitis. Factors that correlate with a poorer prognosis include B symptoms, multiplicity and bilaterality of lung lesions, pleural invasion and cavitation,[7] and Yousem *et al*. have suggested that age greater than 60 years old and clinical relapses also indicate a worse prognosis.

In this current case, the patient was treated with only radiation therapy, because she rejected chemotherapy. The disease has recurred twice, but she is still alive more than 12 years after the initial presentation. However, since the chest CT at the first diagnosis showed a mass with a cavity, we are continuing to observe the patient carefully after treatment.

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