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Primary Pulmonary Hodgkin's Lymphoma with Epstein-Barr and Cytomegaly Virus Infections. A Case Report and Differential Diagnosis

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We report a rare case of primary pulmonary Hodgkin's lymphoma associated with Epstein-Barr virus (EBV) and cytomegaly virus (CMV) infections as demonstrated by *in situ* hybridisation method. Reed-Sternberg cells were CD30 positive. Numerous CD15+ cells were noticed, some of them with concomitant CMV infection.

Introduction

A British physician Thomas Hodgkin first described Hodgkin's disease in 1832. The Reed-Sternberg cells or their variants in a given cellular environment are diagnostic. However, the neoplastic cells make up only 0.1 - 10% of the infiltrate [9]. The origin of these cells is not clear but they are thought to derive from lymphoid cells (probably B or T lymphocytes) [6]. The remaining cells are small lymphocytes, single eosinophils, neutrophils, histiocytes (tissue histiocytes either foamy or epithelioid), plasma cells and fibroblasts [7].

Most patients present a palpable tumour. Most frequently (90%) the primary site is lymph node - in the neck (75%), axilla (15%), groin (10%), mediastinum and paraaortal. Other lymph nodes, especially peripheral are not involved. Lymph nodes enlargement is usually a slow process; they are painless, although pain may occur upon alcohol consumption. Primary extra lymphatic location is very rare, apart from those associated with HIV infection. Secondary extra lymphatic organ involvement is quite frequent. When untreated, this malignancy spreads via the lymphatic and then disseminates in the internal organs (spleen, liver, bone marrow, lungs, pancreas, kidneys, and adrenals) [7].

A Case Description

This female patient J.H., born on 17 November 1948 (age 54, height 163cm, weight 98kg) had had annually bronchitis without radiological signs. Since October 2001 she has been presenting with bronchitis, fever (38˚C) and dyspnoea, at first at exertion, then also at rest. Chest X-rays revealed disseminated, infiltrative lesions of the lungs (especially in the left lung). The patient was admitted to a chest ward for further examination.

CT of the chest was performed after intravenous contrast administration. Bilaterally in lower lobes, irregular infiltrative lesions (alveolar consolidations mainly), not exceeding 30mm in diameter were seen in the subpleural region. Air bronchogram was found in the infiltrate in the anterior segment of the lower left lobe. No pathological changes were seen in the mediastinum and hili. As compared with the previous CT regression of pulmonary infiltrate was detected.

Bronchofiberoscopy revealed bilateral inflammation of the tracheobronchial tree mucosa, especially on the left side in the lower lobe. Oedema was also noticed. Outlets of segmental bronchi were patent but narrow. Abundant white secretion was found, which was sampled for study. Cytological examination of the sputum and bronchial secretion showed the presence of pus, blood and metaplastic epithelia. Streptococcus spp. was cultured from bronchial secretion; fungi were not cultured.

Perfusion scintigraphy of the lungs revealed abnormalities, especially in the lower half of the right lung (various images depending on the body position) - suggesting a thromboembolism. Impaired perfusion of the left lung implied changes secondary to radiological changes. Because of the chronic disease process and obesity of the patient it was not possible to draw unequivocal conclusions with respect to the aetiology.

Spirometric measurements of the flow lung volume revealed a restrictive pattern.

Abdominal ultrasound suggested fatty change in the liver. The spleen was normal.

Due to obstacles in establishing the aetiology surgical biopsy of the lung was performed. A posterolateral left

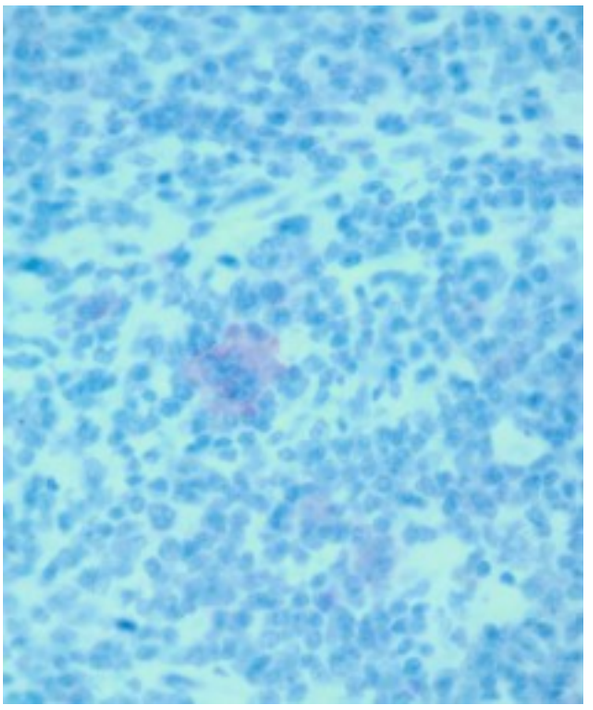

Fig. 1. Immunostaining for CD30 positive in the cytoplasm of Reed-Sternberg cell.

incision was made and the chest was opened through the fifth left intercostal space. In the lower and upper lobe there were hard nodular, irregular lesions with whitish patch on the visceral pleura. Lymph nodes were not enlarged. Wedge resection of the lower lobe involving one peripheral tumour was performed. The tumour was cut and smeared for study by cytology, which did not identify neoplastic cells.

Left lung tissue was obtained for study and fixed in formalin. Some of the specimens were stained with haematoxylin and eosin for study by histology and parallel paraffin sections were immunostained for CD15, CD30 and CMV, using DAKO antibodies according to procedure recommended by manufacturer (Granulocyte-Associated Antigen CD15, Clone C3D-1, dilution 1:25, Ki-1 Antigen CD30, Clone Ber-H2, dilution 1:40, mouse Anti-Cytomegalovirus CCH2+DDG9, ready-to-use; all antibodies from DAKO A/S, Denmark). Other paraffin sections were used for hybridisation *in situ*, which revealed the presence of CMV and EBV.

The whole process of hybridization *in situ* was performed on Bench Mark Discovery Staining Module, Ventana Medical Systems Inc., Tuscon, AZ. Alkaline Phophatase Blue Detection Kit, INFORM CMV, INFORM EBV probes, INFORM Anti-FITC Linker Antibody, Protease I, buffers and other reagents were purchased from Ventana Medical Systems, Inc.

Paraffin-embedded tissue sections were deparaffinised using EZ Prep buffer, then digested with Protease I for 4 minutes. Probes were applied and denaturation was performed at 85˚C for 10 minutes, hybridisation at 37˚C for 1 hour. Probes labeled with fluorescein contained a cocktail of oligonucleotides in a formamide-based diluent. After hybridisation, tissues were washes 3 times using 2xSSC buffer for 6, 2 and 6 minutes at 57˚C (for EBV probe) and at 47˚C (for CMV probe). Incubation with Anti-Fluorescein monoclonal antibody was performed for 20 minutes. Then, Alkaline Phosphatase Blue Detection Kit was applied using standard Ventana MS protocol. As substrate ENHANCED NTB and ENHANCED BCIP were used (incubation for 30 minutes). Tissues were counterstained with Nuclear Fast Red for 10 minutes. Slides were washed with warm tap water with detergent (to remove Liquid Coverslip-LCS) and dehydrated in 70% ethanol (for 30 seconds), 100% ethanol (2 times for 30 seconds), acetone (for 1 minute) and xylene (2 times for 3 minutes). Slides were coverslipped in permanent mounting medium (Consul-Mount Shandon).

By histopathology the changes in the lungs corresponded to Hodgkin's disease. The sectioned lung was totally airless, blurred due to lymphoid infiltration. Single RS cells, H cells and popcorn cells were present. Few mitoses and focal fibrosis were also seen. Lymphoid cells and eosinophils were found in the background.

Immunostaining for CD30 was typically positive in the cell cytoplasm of RS cells (Fig. 1). CD15 positivity was found in small lymphoid cells in the infiltrate and in cells with morphological signs of cytomegaly. HE staining also revealed single cells with intranuclear inclusions characteristic of cytomegaly. Immunostaining for CMV revealed positive reactivity in some Hodgkin's cells. Hybridisation *in situ* confirmed the presence of CMV in these cells and in the cells of bronchial epithelium. Hybridisation *in situ* by means of EBER revealed the presence of EBV in conglomerates of Hodgkin's cells (Figs. 2 - 5).

Discussion

HD may involve practically each organ both as a primary and secondary site. In the chest HD is frequently observed in the thymus, mediastinal lymph nodes and exceptionally in the lungs [3 - 5, 8], which are frequently a secondary location. In 12% of the patients lungs are affected, whereas autopsy examination reveals pulmonary involvement in 58% of cases [4]. Secondary lung involvement occurs as a continuation of the disease process from the mediastinum or in the disseminated form via vascular spread of neoplastic cells. Other organs are usually also involved.

Primary Hodgkin's disease in the lungs differs slightly from primary non-Hodgkin's lymphoma and secondary

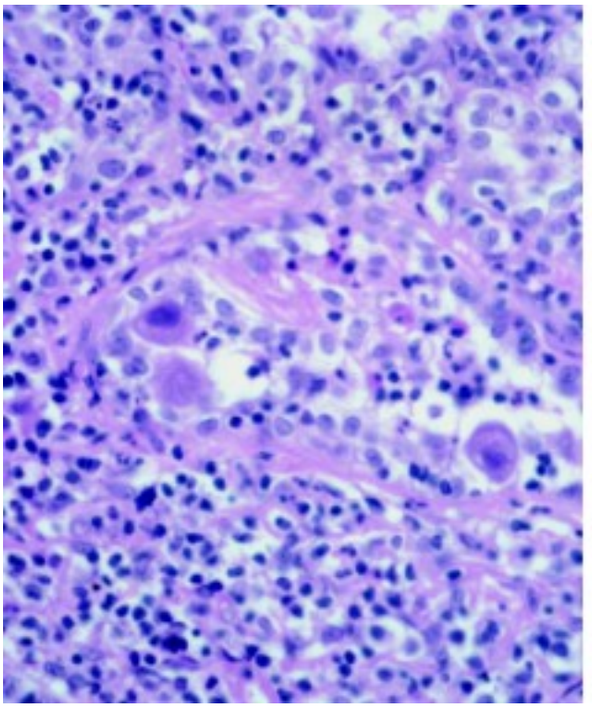

Fig. 2. Single cells with the signs of cytomegaly. HE.

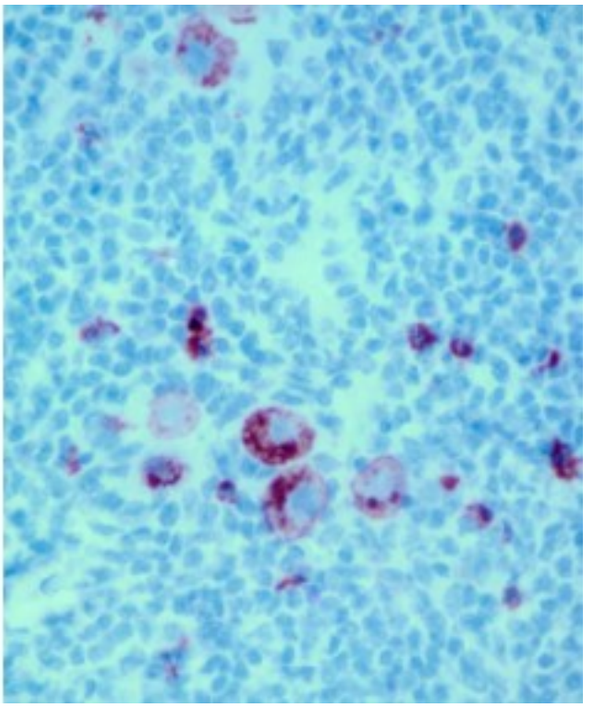

Fig. 3. CD15 positivity was found in small lymphoid cells in the infiltrate and in cells with morphological signs of cytomegaly.

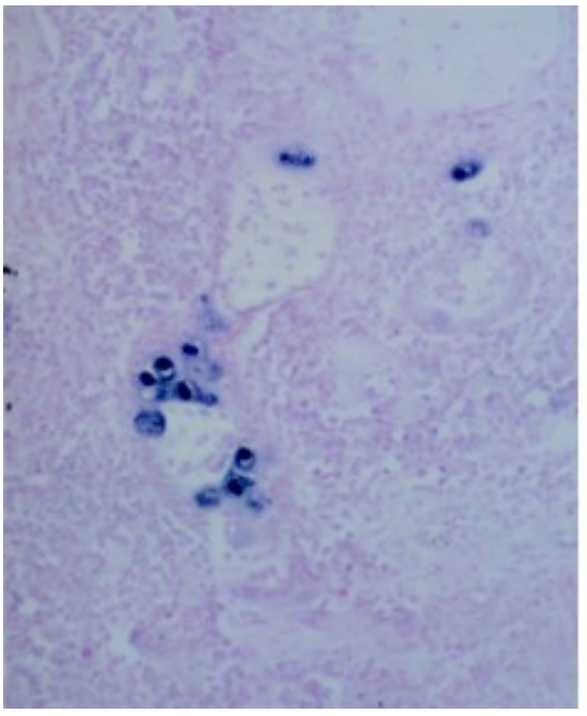

Fig. 4. Hybridisation *in situ* confirmed the presence of CMV in some lymphoid cells and in bronchial epithelium.

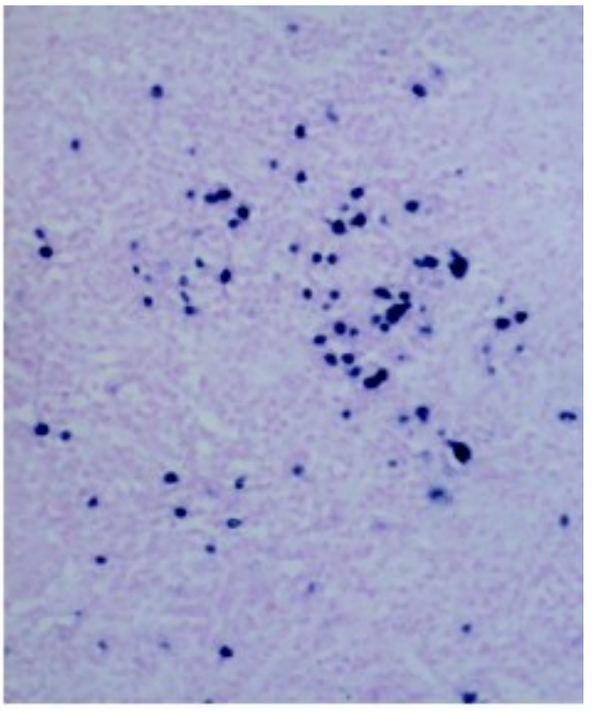

Fig. 5. Hybridisation *in situ* by means of EBER revealed the presence of EBV in conglomerates of Hodgkin's cells.

lung involvement. Nodular lesions (frequently multiple) and cavity formation are more common. A zonal predominance has not been reported in NHL and secondary lymphomas [4]. The following criteria for diagnosing PPHD have been proposed:

- histological pattern typical of Hodgkin's disease;
- lung involvement without or with only slight involvement of hilar lymph nodes;
- no involvement of other lymph nodes [1, 2].

PPHD affects mainly people in the 3rd and 7th/8th decade of life. A female predominance is observed (F:M=1.4:1). Most patients present with general symptoms such as body weight loss, fever, nocturnal sweating. Dry cough, chest discomfort, dyspnoea, haemoptysis, or panting is common. Two-year survival without relapses is observed in less than 50% of the patients. Negative prognostic factors include the involvement of both lungs, pleura or more than one lobe, cavitation, advanced age, multiple lesions and general symptoms on admission [4, 5]. Chest X-rays in 61 cases of PPHD revealed the presence of a mass or tumour in 74%, consolidation within lung tissue in 22% and normal radiological pattern in 3%. Upper lobes are affected twice as frequently as the lower ones. Cavitations are seen in 1/3 of patients [4]. Lung tests may reveal ventilation and perfusion abnormalities with a restrictive or mixed pattern. Restriction may be associated with progressive interstitial fibrosis and/or lymphomatous infiltrate.

NS is the most frequent type of PPHD; LD in this location has not been described [5].

Because of varying histological patterns in HD differential diagnosis includes a wide spectrum of frequently different disease entities from malignant non-haematological neoplasm (carcinoma, melanoma, sarcoma, germ cell tumour) through non-Hodgkin's lymphomas, benign lymphadenopathies to paragranuloma.

Histopathological findings serve as a basis for differentiating HD from carcinoma and melanoma. However, in some cases it may be helpful to immunostain for keratin in carcinoma and S-100 protein and HMB-45 in melanoma. It is noteworthy that some non-haematopoietic neoplasm (for instance adenocarcinoma) may express CD15 [1, 7].

Post-thymic T-cell lymphoma (PTTCL) may resemble Hodgkin's disease by microscopy. However, the former shows a marked cell pleomorphism from small, through medium-sized to large. First differences may also be found in the clinical course of the disease. Other signs corresponding to HD include partial involvement of lymph nodes, necrotic foci surrounded by atypical cells, the presence of noncaseating granuloma, while a large number of mitotic figures imply PTTCL. Occasionally immunohistochemistry and genotyping are necessary. CD45RB expression is present only in 10% of NHL. No expression of CD45RO and CD43 is observed in less than 10% of T-cell lymphomas. CD30 antigen is seen in almost 90% of HD cases and only in 10% of NHL. CD40 is also a good marker, which occurs in 70% of HD and only 13% of NHL. In frozen sections PTTCL cells express a number of antigens common for T lymphocytes. In HD most small lymphocytes have a normal T-cell phenotype, while H cells do not express pan-T-cell antigens or only one or two. The presence of clonal rearrangement of beta-T-cell receptor gene in PTTCL cells may also be a differentiating factor [4, 9].

The cells of ***anaplastic large cell lymphoma (ALCL)*** resemble Hodgkin's cells by cytology. They may also occur in a cell environment similar to HD [2, 9]. In these circumstances the clinical course is a differentiating factor. Lymph node sinuses are more frequently focally involved, which is rare in HD, and doughnut cells are present. Neoplastic cells in ALCL and HD express CD30. However, positive CD45RB, CD43 and CD45RO reactivity is observed in ALCL, whereas CD15 positivity is seen in HD. ALCL cells, in contrast to HD cells are frequently positive for epithelial membrane antigen [2, 7, 9].

Occasionally ***B-cell lymphomas*** (for instance large B-cell lymphoma) may resemble HD, which poses a significant problem, especially in the mediastinum, where both entities are frequently located. Histologically, T-cell rich and histiocyte rich lymphomas as well as B-cell immunoblastic lymphoma may be difficult to differentiate. By immunohistochemistry B-cell lymphomas are almost always CD45RB+ and CD20+, expressing CD15 in only 5%. CD20+ cases make up about 20% of HD cases. In frozen sections B-cell lymphomas in contrast to HD show the presence of immunoglobulins with monoclonal restriction of light chains. If these immunoglobulins are not expressed, staining for CD20, CD19 and CD22 present in almost all B-cell lymphomas is usually decisive [4]. Differential diagnosis must also include small lymphocytic lymphoma, which resembles LR type, and viral lymphadenopathies, including mononucleosis. In the latter case the clinical manifestations and course of the disease are helpful. Tonsillar involvement is frequent in mononucleosis but rare in HD. Atypical lymphocytosis in peripheral blood also implies viral origin of lymphadenopathy. In the course of viral infection lymph nodes may contain cells resembling RS cells; however other histological signs usually help in differentiation from HD. Some diagnostic difficulties may also occur in cases of cat's scratch disease and other necrotising granulomatous diseases [2, 5].

Unfortunately environmental factors, which affect the development of HD are not well known. Probably EBV infection plays the most important role. There is evidence

suggesting that young adults and middle aged subjects have HD as a result of EBV infection. However, it is a rare complication of EBV infection and it depends on the age of infection onset. Nevertheless this virus is present in 1/3 to 1/2 of HD cases. In these circumstances other genetic, environmental etc. factors must play a role in the pathogenesis of HD. Some investigators suggest that all HD patients have primary EBV infection, however in subjects with unaltered immune response the viral genome may be lost in the infected cells. For this reason part of HD cases would be EBV negative. Nevertheless the relationship between EBV infection and HD seems obvious. The oncogenic EBV virus has already been identified as a triggering factor in the development of Burkitt lymphoma or nasopharyngeal carcinoma [6, 10].

The elevated titter of anti EBV antibodies can be observed many years before the development of HD. In about 1/2 of patients Reed-Sternberg cells were found to contain a monoclonal EBV genome and EBV infection preceded clonal expansion of neoplastic cells. In all cases the virus was latent. In our patient hybridisation *in situ* by means of EBER revealed the presence of EBV in conglomerates of HD infiltrates [2, 6, 7, 9].

Frequent lymphocytopenia and altered cellular response in patients with HD predisposes to opportunistic viral infections. This fact may account for the presence of CMV infection as a result of superinfection in our patient.

The present case confirms various clinical presentations of Hodgkin's disease.

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Errata to Pol J Pathol 2002, 53, 4

In the paper: P. Dzięgiel, P. Surowiak, J. Rabczyński, M. Zabel "Effect of Melatonin on Cytotoxic Effects of Daunorubicin on Myocardium and on Transplantable Morris Hepatoma in Rats", the photographs - Fig. 1, 2 and 4 on the page 202 were erroneously placed. Their appropriate location should be as follows:

Fig. 1. → Fig. 2.
Fig. 2. → Fig. 4.
Fig. 4. → Fig. 1.

The editorial staff apologizes to the authors for the error.

Announcement

The Congresses of Veterinary Pathology will take place:

- in Dublin (Ireland) – 10-13 September 2003
  21st Congress of European Society of Veterinary Pathology
- in Olsztyn (Poland) – 14-17 September 2004
  22nd Congress of European Society of Veterinary Pathology

Topic problems are:

- veterinary pathology
- experimental pathology
- toxicopathology

Persons, who are interested in attending the Congresses may obtain more information under:

vetpathoff@ucd.ie – e-mail for the Congress in Dublin
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